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THE EFFECTS OF HIGH VOLTAGE CATHODE RAYS ON THE GERMINAL EPITHELIUM OF THE RAT*

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The biologic action of cathode rays has been studied in various laboratories since Coolidge, in 1926, devised a tube which made it possible to observe the effects of the cathode rays outside the apparatus at extremely high voltages. For several years previously, however, Pauli and his co-workers had been making fundamental contributions on cellular alterations in plants, bacteria and wounds, and in the skin of cold-blooded and warm-blooded animals. The extent of their work was limited somewhat by the low maximal voltage which their modified Lenard tube could withstand.

Some practical applications of cathode rays have also been made by Baensch and Finsterbusch[1] in Leipzig, who reported success in the treatment for lupus, tuberculosis, verrucosa cutis, psoriasis, infected superficial wounds and cancroids. They used 95 kilovolts, 3 milliamperes, at 5 cm. distance for from fifteen seconds to two minutes. They urged caution in the use of cathode rays because of the lack of knowledge of the biologic effects on the operator and patient.

Guyer and Daniels[2] studied the effects of cathode rays on experimental cancers in rats. They found that with tumors not too far advanced the first irradiations had a detrimental effect, but that successive irradiations probably stimulated the tumor. They estimated the maximal penetration of the effective rays into living cancers as 0.5 cm. *Drosophila* eggs were killed in five-tenths second or less. The lethal period of exposure for a normal rat irradiated over the belly was from three to four minutes. There was no tendency toward formation of

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1. Baensch, W., and Finsterbusch, R.: Unsere klinischen Erfahrungen mit der therapeutischen Anwendung von Kathodenstahlen, München. med. Wchnschr. **51**:2171, 1927; Weitere klinische Beobachtungen bei der Behandlung mit Kathodenstrahlen, Klin. Wchnschr. **15**:681, 1928.

2. Guyer, M. F., and Daniels, F.: Cancer Irradiation with Cathode Rays, J. Cancer Research **12**:166, 1928.

cancer in the rat's skin. The action of the cathode rays on tumor was regarded as direct and not systemic.

Stenstrom, Watkins, Nurnberger and Fallon,[3] also using the Coolidge cathode ray tube, observed the effects of irradiating the rabbit's ear on the leukocytic formula. The immedite reaction was an absolute increase in both neutrophils and lymphocytes, the numbers returning to normal in a few hours. A sustained reaction of slight absolute increase in neutrophils and a relative and absolute decrease in basophils and monocytes after two days were found.

The genesis of roentgen rays occurs when cathode rays impinge on matter, the spectrum varying with the composition of the anodal target. These roentgen rays, in turn, on striking tissue or some other form of matter give rise to secondary cathode rays. The amount of roentgen rays produced by cathode rays of 200,000 volts, using a target as efficient as one composed of tungsten, is relatively minute; hence the roentgen rays generated by cathode rays striking animal soft tissues, which are largely composed of carbon, hydrogen, oxygen and nitrogen, elements of low atomic weight, would be of exceedingly smaller amount, although very penetrating. Any changes observed in the short distance of the thickness of the rat's skin and underlying organs would in all probability be due to the primary cathode rays, and any differences in effect as seen in the various types of tissue should be explainable on the basis of differences in tissue susceptibility. Studies directed at finding proof of this concept, in biology, may seem chiefly of academic value, but the work of Pauli and his group, of Coolidge and his assistants and of Baensch and Finsterbusch indicates that cathode rays have some definite therapeutic applications, although to the present time they have been found, as a whole, inferior to roentgen rays. Nevertheless, the making available in this manner of so tremendous an electric potential may lead to discoveries of more pronounced value.

In February, 1928, we reported from this laboratory the changes occurring in the abdominal skin of the white rat following exposure to cathode rays.[4] The voltages used were 100,000, 200,000 and 350,000. The current was 1 milliampere. The animal was placed 2.5 cm. from the anodal window and was entirely protected by copper foil, except for the small area irradiated.

It was found that the intensity of the reaction in the skin varied according to the voltage and the length of exposure to the cathode

3. Stenstrom, W.; Watkins, C. H.; Nurnberger, C. E., and Fallon, M.: The Influence of Cathode Rays on Leukocytes of Rabbits, Proc. Soc. Exper. Biol. & Med. **26**:380, 1929.

4. Jacobsen, V. C., and Waddell, K. C.: The Effects of High-Voltage Cathode Rays on the Skin of the Rat, Arch. Path. **5**:195 (Feb.) 1928.

rays. Marked gross changes were produced: discoloration, depilation, congestion, edema, later ulceration and rather sluggish healing of the ulcer.

Microscopically, the most characteristic lesion was found to be a fusing of the collagen fibers of the corium and necrosis of the epithelium of the skin and hair follicles. Altered staining properties indicated profound chemical changes with the production of an acid reaction. The vascular injury was not severe and occurred after the hyalinization of the collagen. The depth of penetration of the primary cathode rays as measured by the thickness of the zone of fused collagen varied from 0.07 to 0.22 mm., as seen in the stained section. Allowing for shrinkage of the tissue in the various fixing and dehydrating fluids, one concludes that the penetration was probably about twice that depth.

The peculiar degeneration of the collagen is apparently similar to that caused by exposure to roentgen rays; hence the evidence was regarded as confirmatory of the hypothesis that the biologic effects of roentgen rays are not due primarily to the roentgen rays but to the cathode rays which the roentgen rays produce in the tissues.

One of the goals of research in pathology is to discover in organs, tissues or their component cells alterations which are characteristic or pathognomonic for a given condition. The macroscopic lesions in many diseases are well known and almost as constant as the normal anatomic format. Also, the microscopic picture of cell arrangements and certain finer cell changes can be included in the category of well established anatomic accompaniments of many diseases.

It is quite as essential to identify what are beyond reasonable doubt the characteristic or unique effects of the various forms of light. The lesions caused by roentgen rays in human and lower animal tissues have been repeatedly studied by numerous investigators ever since Roentgen's original discovery. A characteristic single effect is acute hyaline degeneration of collagen fibers, as pointed out by Wolbach [5] and others. In addition, there are obliterative vascular reactions, hyperplastic epithelial, later atypical epithelial, changes and the production of skin cancer.

Chromatin is greatly affected by roentgen rays, but not in a manner that permits a diagnosis of roentgen injury solely on the basis of structural alterations in chromatin. It is possible, however, to damage the nucleus profoundly without injuring the cytoplasm; for instance, in vitro, heart muscle of an embryonic chicken will continue to beat for days after the nucleus has been killed by exposure to a lethal

5. Wolbach, S. B.: A Summary of the Effects of Repeated Roentgen Ray Exposures upon the Human Skin, Antecedent to the Formation of Carcinoma, Am. J. Roentgenol. **13**:139, 1925.

dose of radium.[6] Trypanosomes so exposed may retain their motility, but cannot divide.[7]

Recently, Cramer [8] suggested that in death from roentgen irradiation destruction of the organs of the reticulo-endothelial system has taken place. Organs characterized by the capacity to absorb dyes during life are likewise affected directly by irradiation. He saw an analogy between the action of histamine and the intoxication due to roentgen rays in the vagotonia, acidosis and leukocytic formula of the latter condition. The clinical picture of intoxication due to roentgen rays is not clearcut and only adds further stress to the assertion that in the present state of knowledge one knows few absolute criteria for diagnosing a "burn" by the roentgen rays, whether it is in the skin or in a deeper organ.

The male and female germ cells are exquisitely sensitive to roentgen rays. Perthes,[9] in 1903, was the first to point out the relationship between the activity of reproduction in tissues and the degree of radiosensitivity. He worked on developing eggs. In 1906, Bergonié and Tribondeau,[10] following work along similar lines, formulated a statement or "law" which is as follows: "Les rayons X agissent avec d'autant plus d'intensité sur les cellules que l'activité réproductrice de ces cellules est plus grande, que leur devenir karyokinétique est plus long, que leur morphologie et leurs fonctions sont moins définitivement fixées." (The x-rays act on the cells with such intensity that the reproductive activity of the cells is greater, their karyokinetic growth is longer and their morphology and functions are less definitely fixed.)

Our previous work on the skin of the white rat [4] indicated that at least one supposedly characteristic effect of roentgen rays (acute hyaline degeneration of collagen) could be produced by the direct action of electrons or cathode rays of high velocity. It was therefore believed advisable to study the reactions of the more sensitive germinal epithelium to cathode rays. The testes of the rat are readily available for such an experiment, but since they are covered by skin any changes in them would have to be explained as due to their being more sensitive than the unaffected layer of subcutaneous connective tissue below the level

6. Prime, F.: The Action of Radium on Embryo Heart Muscle, Proc. New York Path. Soc. **16**:56, 1926.

7. Halberstaedter, P.: Experimentelle Untersuchungen an Trypanosomen über die biologische Strahlenwirkung, Berl. klin. Wchnschr. **51**:252, 1914.

8. Cramer, H.: Studien zur biologischen Wirkung der Röntgenstrahlen, Strahlentherapie **28**:431, 1928.

9. Perthes: Versuche über den Einfluss der Röntgenstrahlen und Radiumstrahlen auf die Zellteilung, Deutsche med. Wchnschr. **30**:668, 1904.

10. Bergonié, J., and Tribondeau, L.: Interpretation de quelques resultats de la radiotherapie et essai de fixation d'une technique rationelle, Compt. rend. Acad. d. sc. **143**:983, 1906.

of the upper 0.2 to 0.4 mm., which was the "zone of effect," as determined by our previous experiments on the rat's skin.

TECHNIC OF THE EXPERIMENT

In a previous publication from this laboratory was described a method of exposing a rat in front of the anodal window of the Coolidge cathode ray tube. The experiments to be reported here were performed in the same manner except that the testes were exposed rather than the abdominal skin, that is, the scrotum containing the testes. The animal was tied to a board, the scrotum being 2.5 cm. from the anode. The rest of the animal was covered with copper foil. The scrotum was shaved before exposure, because a thin covering of hair is sufficient to stop many of the rays. The shaving was usually done several hours before the experiment in order that any hyperemia due to the friction involved in the shaving process would have time to subside. At first, only one testis was irradiated, the other being used as a control, this method being fairly satisfactory as a whole. Difficulty was encountered, however, at times, in preventing some exposure of the control side because of the spasmodic movements of the animal.

TABLE 1.—*Protocol of the Experiments*

Number of Rats	Time of Exposure, Seconds	Voltage
3	0.5	200,000
6	1.0	200,000
8	5.0	200,000
4	10.0	250,000
4	15.0	250,000
3	20.0	250,000
3	30.0	250,000
6	Normal controls	

Also there was histologic evidence in a few rats of a "spreading" effect, that is, cathode ray changes could be detected on the medial side of the control testis where it was close to the exposed testis. Totally unirradiated animals of the same age and condition and from the same pens were regarded as more satisfactory controls. Hence in the later work both testes were irradiated.

The actual irradiation was done in the research laboratory of the General Electric Company in Schenectady, N. Y., with the cooperation of Dr. W. D. Coolidge and the invaluable technical assistance of Mr. C. N. Moore.

Thirty-seven adult white rats were used in this investigation. They were about 5 months old and apparently healthy and virile. The voltages used were 200,000 and 250,000, about equally divided as to number of animals. The current was 1 milliampere. In no instance was an anesthetic used, nor was the testis ever exposed directly. It was thus possible to study also the cutaneous changes of the scrotum and to compare them with the cutaneous reactions of the abdominal wall as previously reported.

OBSERVATIONS

Immediately after the irradiation, the hairs bordering the shaved zone were seen to have turned a definite yellow. There was also some edema, with vascular dilatation, which became marked in twenty-four hours. The severity of the reaction seemed roughly proportional to the voltage and time of exposure. The irradiation was accomplished in

interrupted exposures of one second each with about one-half second intervals.

The superficial epidermis began to desquamate in forty-eight hours, and considerable drying of the skin occurred. Intracutaneous hemorrhage and edema became more marked, but no definite ulcer with moist surface developed. It seems unlikely from gross appearances that infection played any important rôle in the subsequent events.

The animals which were subjected to five-tenths second and one-tenth second exposures had slight local reactions, and after the superficial flaking off of loosened epithelium and a mild edema the skin in

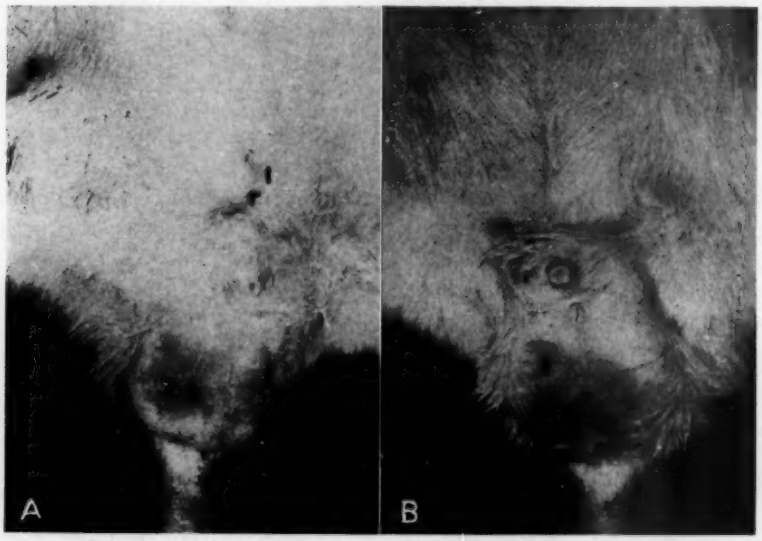

Fig. 1.—*A*, scrotal area (rat 42) twenty-five hours after irradiation for fifteen seconds at 250,000 volts, showing edema of the scrotum and some discoloration with hemorrhage into the skin.

B, scrotal area (rat 43) twenty-five hours after irradiation for thirty seconds at 250,000 volts. The cutaneous edema and hemorrhage are more marked than in rat 42.

about eight days had regained its normal texture, although the hair was not so abundant as before.

The rats irradiated for one second were killed with a blow on the head, six hours, six days, eight days and forty-nine days later; those given five seconds' irradiation, six days, eight days and forty-nine days later; those given ten seconds' exposure, after seventy hours, eight days and fifty days; those given fifteen seconds' exposure after twenty-five hours, four days, twelve days and twenty-one days; those given twenty seconds' exposure, after eight days; those given thirty seconds'

exposure, after twenty-five hours, twelve days and twenty-one days, respectively. Six animals were used as controls. The series, then, seems sufficiently representative of time intervals to give a reasonably comprehensive picture of the alterations in the testis under the conditions of the experiment.

In no instance was there observed any adhesion of the subcutaneous tissues to the tunica vaginalis. The testis when exposed in its sac was always smooth and glistening. In the rats given five-tenths, one-tenth,

Fig. 2.—Scrotal area (rat 39) eight days after irradiation for twenty seconds at 250,000 volts. A large necrotic and hemorrhagic area is present in the scrotum with slightly dry surface.

five and ten seconds' exposures, no gross changes were observed. In those given fifteen, twenty and thirty seconds' exposures, there was frequently, but not always, present a zone of translucency directly beneath the irradiated area, and occasionally punctate hemorrhages. With the heavier doses, there was visible edema of the tunicae, but no hydrocele. The epididymis showed no macroscopic changes. The testes when incised bulged out in a semi-diffluent manner, but this is the normal consistence in the white rat. The tissue was fixed in small portions in Zenker's fluid, neutral solution of formaldehyde-Zenker, a diluted

solution of formaldehyde, U. S. P. (1 : 10), and according to Kolachev's osmic-bichromate method for the demonstration of the Golgi apparatus and lipoid and fat droplets.

Before describing the abnormalities encountered in the testes of the white rats exposed to cathode rays, I must point out that in the so-called normal testis not only of the rat but of other mammals various degenerations are encountered. Barratt and Arnold,[11] in their study of lesions in the rat's testis caused by roentgen rays, described the following changes in their normal series: (1) necrosis of spermatocytes of the first order in a small fraction of animals, the cells taking basic instead of acid dyes, and being found both in cells undergoing mitosis and in those not dividing; (2) fatty globules near the

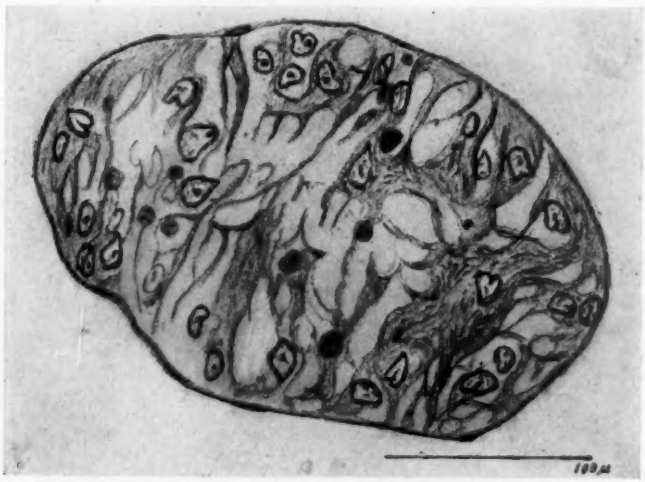

Fig. 3.—Seminiferous tubule thirteen days after the application of roentgen rays (copied from Barratt and Arnold: Arch. f. Zellforsch. **7**:242, 1911-1912). All seminal cells have disappeared. Externally, the basement membrane is seen. Distributed within the tubule are numerous nuclei of cells of Sertoli the cytoplasm of which forms a coarse framework, within the meshes of which fatty masses, more or less darkly stained with osmic acid, are sparsely scattered. No definite lumen is present in the tubule (compare with fig. 7).

lumen of tubules, about 3 microns in size, in the cytoplasm of spermatids and in cast-off particles of cytoplasm, a dozen or more in a group; (3) fatty globules from 2 to 12 microns in size in young spermatids.

These changes were seen chiefly in the noncopulatory periods, when the testes were in the abdomen. Multinucleate cells rarely occurred and occasionally enlargement of archoplasmic vesicles. They did not encounter intranuclear body vacuoles or irregular mitoses.

11. Barratt, J. O. W., and Arnold, G.: Cell Changes in the Testis Due to X-Rays, Arch. f. Zellforsch. **7**:242, 1911-1912.

In my control animals, few degenerative changes were seen in numerous sections from both testes. No cell necroses were found, nor any multinucleate or giant cells of any type. Fatty globules, however, were not infrequently seen lying free in the lumen of tubules and sometimes in the spermatid zone. They were minute, and their significance was not clear.

The following observations were made on tissue fixed in Zenker's fluid and stained with hematoxylin-eosin and eosin-methylene blue.

One exposure of one-half second, at 200,000 volts, produced definite changes in the skin, and in six days repair was well under way beneath the zone of necrosis. Epithelial regeneration was definitely retarded, however. The tunica vaginalis testis was normal. The lymphatics

Fig. 4.—Seminiferous tubule eleven days after the application of roentgen rays (copied from Barratt and Arnold). The seminal cells have disappeared, their place being taken by numerous fatty masses stained in varying degrees of darkness by osmic acid. The nuclei seen are those of cells of Sertoli, the cytoplasm of which forms a loose meshwork throughout the tubule. The lumen of the tubule has become obliterated.

beneath the tunica were dilated. Blood capillaries occasionally showed endothelial proliferation, but no thrombosis. The seminiferous tubules were all intact. The interstitial cells appeared normal. The Sertoli cells and spermatogonia were unaffected. Many tubules showed hyaline droplets lying free in the midzone and also free in the lumen intermingled with mature sperm. These were probably seminal granules derived from degenerated spermatids. A few balloon-like vacuolated cells in some tubules along the periphery of the testis were probably degenerated spermatocytes of the first order.

Irradiation for one second gave a picture much like the foregoing, except that hyaline droplets were more numerous after six days. Often it appeared as though they represented altered heads of spermatozoa, the flagella having disappeared. Pyknosis in cells of spermatid type was prominent in some rats. An occasional atrophic tubule was found, but this could well be unrelated to the irradiation.

Irradiation for five seconds produced marked degeneration of the scrotum, but there was no distinct continuity of lesion between the subcutaneum and the tunica vaginalis. Lymphatic dilatation and some endothelial proliferation were present. Along the periphery of the testis, just beneath the scrotal skin, a few tubules were encountered in which the mature spermatozoa showed marked swelling of their heads, which took a pale basic stain. No tails could be demonstrated on many sperm.

The results in the series of rats given five seconds' exposure to 200,000 volt cathode rays may be summarized as follows. The tunica vaginalis was normal. The lymphatics were dilated. The tubules just beneath the capsule showed swelling and vacuolation of spermatids, with desquamation and fragmentation of cells. The Sertoli cells were normal. A dearth of mitotic figures in spermatogonia was noted. It was at times difficult to be certain of the identity of the many degenerated cells. Hyaline droplets were abundant in the lumen. Some phagocytosis of spermatozoa by large mononuclear cells was noted. The interstitial cells were normal and often contained a lipoid pigment. The aforementioned alterations were visible in six days and as late as forty-nine days after exposure could still be detected. The number of tubules affected was small, but their location beneath the field of irradiation would seem to indicate that the changes were probably due to the irradiation. Most of the tubules showed abundant mitoses and spermatogenesis. The epididymis was usually full of sperm, but some cells of the lining membrane were swollen and vacuolated or undergoing a hyalovacuolar change.

So far the evidence of injury of the testis by the cathode rays has been entirely microscopic. However, with exposures of ten seconds and longer there were also gross alterations. Within twenty-four hours, areas of translucency appeared just beneath the capsule on the irradiated side, and occasionally punctate hemorrhages after fifteen or twenty seconds' exposure. The translucent foci were due to degeneration of tubules, the spermatozoa and most of the epithelium of which had undergone necrosis with disappearance of most cells after seven days. That it takes much less irradiation to produce effects in germinal epithelium than in connective tissues was evident in this series of animals. The tunica vaginalis and the underlying fibrous capsule showed no histologic change, while the seminiferous tubules were con-

siderably altered. In some rats, a zone about five tubules, or about 0.6 mm., deep was affected, with a sharp line of demarcation between healthy and damaged tubules; that is, whole tubules were injured usually. In the rats exposed for fifteen and twenty seconds, the depth of the injury was slightly greater, and there was a more gradual diminution of visible lesions.

The sequence of changes seemed to be, first, swelling and vacuolar degeneration affecting the spermatids and spermatocytes. The sperma-

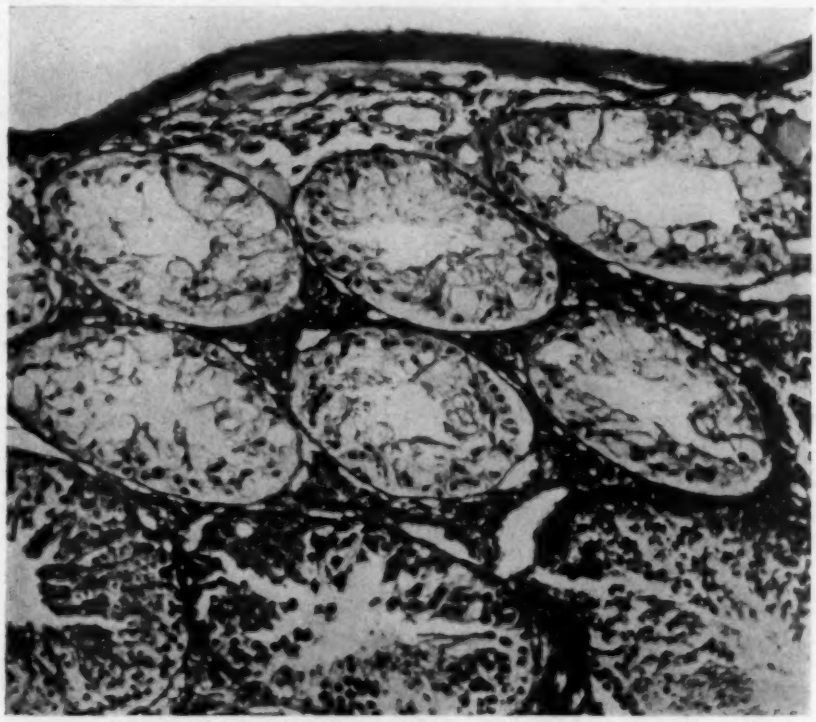

Fig. 5.—Spermatic tubules to a depth of about 0.3 mm. in rat 38 after fifteen seconds' irradiation of scrotal area at 250,000 volts, showing loss of spermatogenic cells and proliferation of Sertoli's cells; × 154.

tozoa seemed more resistant. After four days, the damaged cells and spermatozoa had disappeared, and only shadowy outlines of cells remained. Even Sertoli's cells had been destroyed in some tubules. The changes in the nuclei were largely due to chromatolysis with or without preceding pyknosis. Intranuclear vacuoles were found in spermatocytes and spermatids. After fifty days, large multinucleate phagocytic cells appeared and engulfed nuclear particles and heads of spermatozoa. Sertoli's cells almost filled the lumen of some tubules.

Obliterative proliferations of vascular endothelium were occasionally seen. In marked contrast to the tubules within the "zone of influence," other tubules in the same low power field appeared normal with spermatogenesis in progress and mitoses in about normal number and type.

The profound alterations in the germinal epithelium described were visible with the ordinary methods of fixation and staining. It was thought that finer cell changes might be detected by the use of other

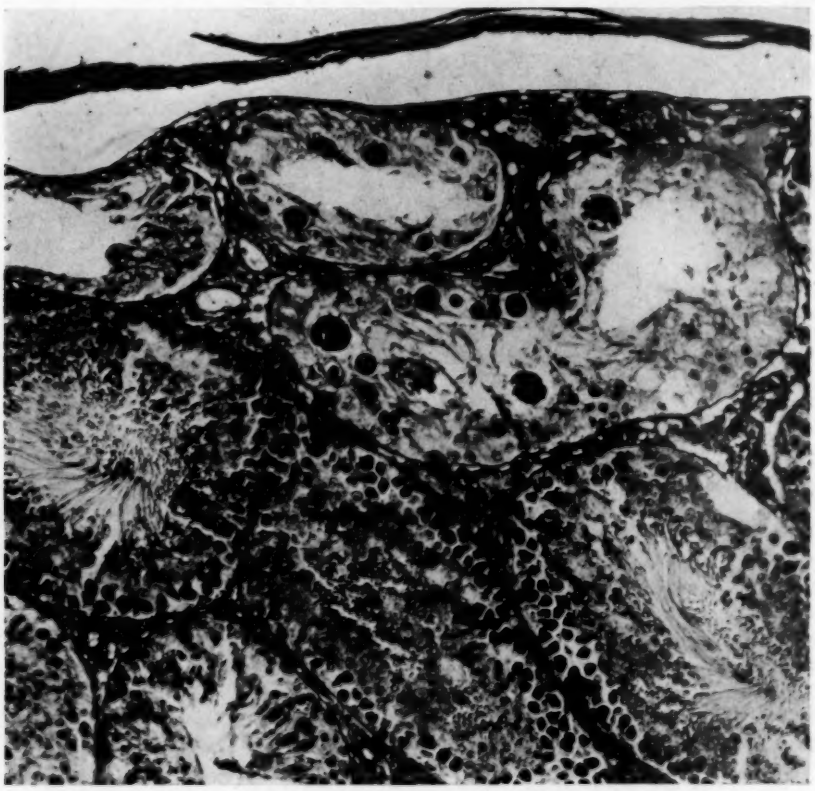

Fig. 6.—Superficial tubules of testis in rat 39 eight days after twenty seconds' irradiation of scrotal area at 250,000 volts, showing destruction of spermatogenic cells; many multinucleate cells, some phagocytic, in two of the tubules; proliferation of Sertoli's cells, which tend to fill the lumen, and nuclear degeneration in many of Sertoli's cells; × 163.

technics. Special attention to certain structures in cytoplasm and nuclei seemed warranted. Gatenby and Wigoder [12] recently studied the effects of roentgen rays on the Golgi apparatus of the epithelium of the guinea-

12. Gatenby, J. B., and Wigoder, S.: The Effect of X-Radiation on the Spermatogenesis of the Guinea-Pig, Proc. Roy. Soc. **104**:351, 1928.

pig's testis. Gatenby had previously concluded that the centrosome was not injured by roentgen rays, that strong doses prevented neither proper orientation of the spermatids in relationship to the lumen nor proper outgrowth of the flagellum from the head centrosome. Gatenby and Wigoder summarized their work as indicating that the specific effect of roentgen irradiation on cell mitosis is due to a temporary breaking down of certain lipoid substances necessary for mitosis. This lipoid substance was supposed to be located in the cortex of the Golgi

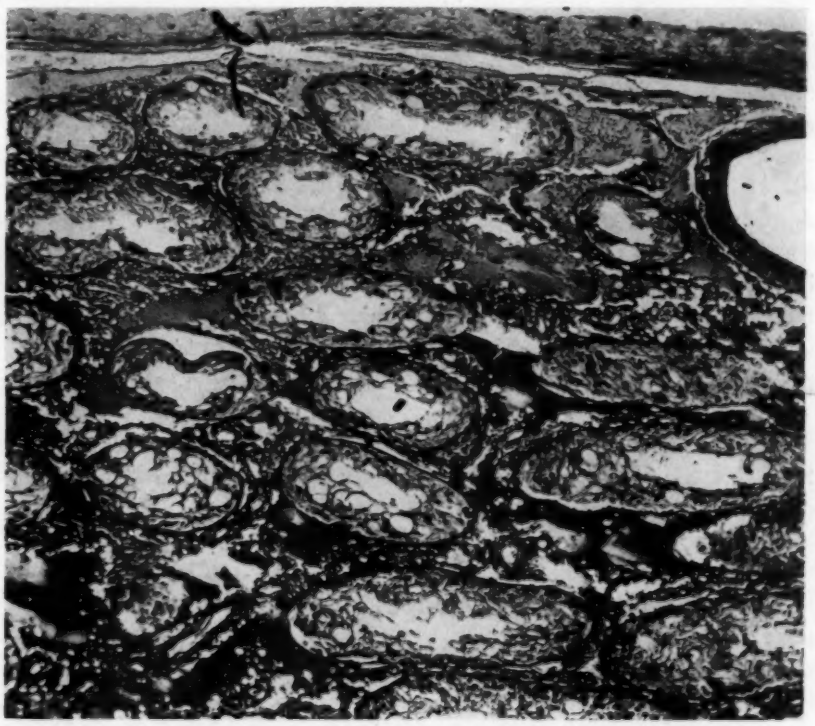

Fig. 7.—Degeneration of seminiferous tubules to depth of about 0.6 mm. (×138) in rat 41 fifty days after ten seconds' irradiation of scrotal area at 250,000 volts. Sertoli's cells tend to fill up the lumina and themselves show many changes. There is dilatation of the lymphatics. The tunica vaginalis is normal. Vacuolation and fatty degeneration are prominent. Compare with figure 3.

apparatus. They found that sometimes the Golgi lipoid cortex became flocculent and granular instead of smooth. The most radiosensitive period of mitosis was just before the prophase, since it was impossible for the cell at that stage to recover from the effects of broken down lipoids, an abnormal mitosis leading to death resulting. Cells entering mitosis might be killed by inability of the centrosome to pass out of

the idiozome preparatory to amphiaster formation. Postnuclear bodies were sometimes abnormal in the irradiated testis.

The rats in my series exposed for ten, fifteen, twenty and thirty seconds to rays, at 250,000 volts, and a current of 1 milliampere, were killed by a blow on the head. The testes were removed at once, and portions placed in various fixatives. Small pieces were fixed according to the Kolachev-Nassanow method for the demonstration of the internal reticular apparatus of Golgi, an osmic acid treatment combined with bichromate-chromic acid fixation. The Golgi apparatus becomes blackened by this method as do fat droplets and lipoid bodies to a certain extent. It is best to emphasize that errors in technic are easily committed in staining the Golgi apparatus, the most accurate observations of it being made by biologists who have had considerable experience with the various technics. The material may be demonstrable by one method and not by others, hence it is usually advisable to try several before pronouncing on the characteristics of the Golgi apparatus. It is generally considered that the Golgi apparatus is relatively easily demonstrated in the testis by osmification, and Kolachev's technic was used successfully by Gatenby and Wigoder in their studies of roentgen changes in the guinea-pig's testis; hence the observations to be reported here are based on this method.

In the control animals, the Golgi apparatus was usually well shown; hence, I feel, notwithstanding my small experience with this cell structure, that the changes to be described were real in the sense of at least occurring ante mortem and not being due to operative trauma or autolytic processes.

The tubules that showed the most degeneration with ordinary staining methods disclosed varying degrees of alteration of the Golgi apparatus. Fragmentation with dispersion of the particles rather widely throughout the cytoplasm was a common picture, although where the nucleus had been destroyed no trace of Golgi material was found. The cells of Sertoli appeared the least affected as judged by the morphology of the Golgi apparatus.

The acrosome of the spermatid was markedly enlarged twenty-four hours after thirty seconds' irradiation, and the Golgi material often appeared more or less fused with it. The nucleus was frequently surrounded entirely by a ring of blackened fine granules, even in cells that were normal otherwise. In spermatocytes similar changes were noted. More normal-appearing Golgi networks were seen in the more deeply situated tubules indicating that the penetration of the rays was not deep. Some spermatogonia showed a Golgi apparatus resembling a spherical vesicle with a central clearer zone. This might be normal as to shape, but often seemed considerably increased in size.

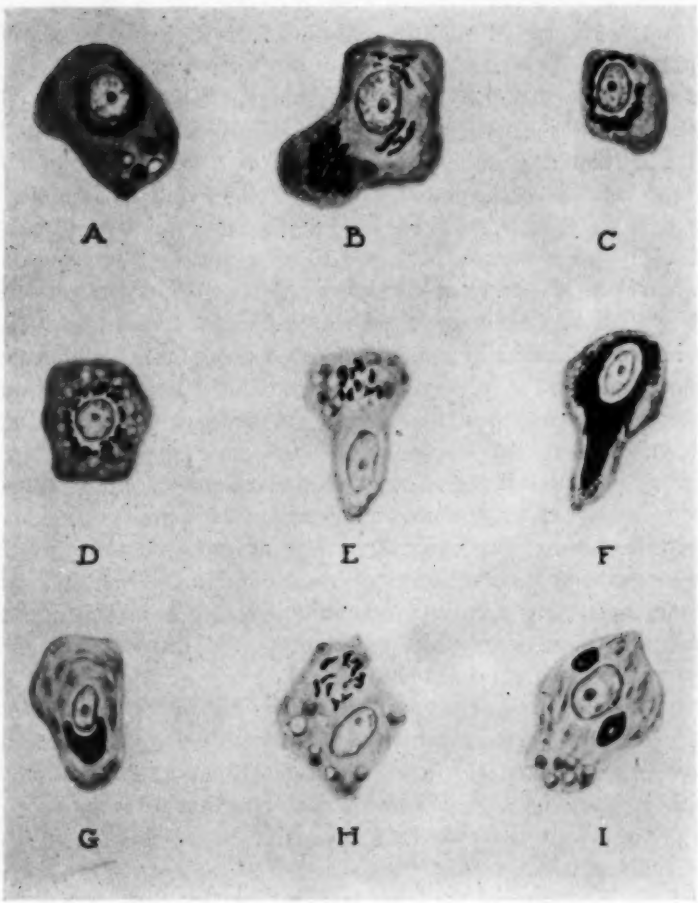

Fig. 8.—Drawings of various cells of the rat's testis following exposure to cathode rays. Kolachev's osmic acid technic was used for the demonstration of the Golgi apparatus. *A*, *C* and *D*, spermatocytes of the first order showing perinuclear arrangement of Golgi material in rat 43 after thirty seconds' irradiation of the scrotal area at 250,000 volts; in *D* fragmentation has occurred. *B*, an osmiophilic network and rodlike and granular bodies isolated from a larger mass lying close to the nucleus. *E*, a spermatid with fragmented Golgi material. *F*, a cell from the midzone of a tubule, possibly representing fused Golgi and acrosomic material. *G*, probably a spermatid, from rat 42 twenty-five hours after irradiation for fifteen seconds at 250,000 volts, showing enlarged archoplasmic vesicle and no definite Golgi network. *H*, fragmented Golgi material; numerous lipoid droplets (rat 42). *I*, cell of doubtful identity, showing separation of Golgi material into two portions, each somewhat spherical and vesicular.

The interpretation of alterations of what might be considered the normal type of Golgi network for a given cell must be accompanied by many qualifications. Cowdry,[13] in particular, emphasized the need of great caution in evaluating such changes. He found the term "apparatus" objectionable because it carries with it the idea of a mechanism. He stated that in *Metazoa,* taken generally, the Golgi apparatus shows a tendency to occur in isolated rods and spherules instead of complicated networks, this being particularly true in stages of oogenesis and spermatogenesis. Occasionally, isolated fragments are met with in *Protozoa,* some writers hence using the term Golgi "body." This, however, gives the impression of distinctive form and to some extent of solidity, as contrasted with the relative fluidity of the Golgi material.

Hyman[14] in his study of the spermatogenesis of *Fasciolaria* found that the Golgi apparatus of the germ cells and certain somatic cells occurs in the form of tiny spherule-like vesicular bodies with a central "chromophobe" and a peripheral "chromophil" substance. The more complicated networks are apparently encountered higher up in the animal scale.

Cowdry[13] reviewed the literature on the morphology of the Golgi apparatus under various abnormal conditions which included tumors, tuberculosis, rabies, experimental poisoning and vitamin deficiency. The most common observation was fragmentation. Dispersal throughout the cytoplasm, perinuclear formations, increase in size, reduction in size and reversal in position are reported, but no one change pathognomonic of a given condition.

In my rats exposed to cathode rays, swelling was a common abnormality of the Golgi apparatus, also fragmentation and perinuclear arrangements. Legendre[15] in 1910 electrically stimulated the posterior root lumbar ganglion of a dog and found fragmentation with dispersal to the periphery of the cytoplasm. Cajal[16] found that autolysis produced fragmentation.

That the exposure of the rat's testis to cathode rays causes definite changes in the Golgi apparatus of the germinal epithelium is suggested by my experiments. Gatenby and Wigoder found that in the testis of the guinea-pig irradiated with roentgen rays the lipoid cortex becomes flocculent and granular. The other degenerative and necrotic changes seen in the testis of the rat exposed to cathode rays seem very

13. Cowdry, E. V.: General Cytology, Chicago, University of Chicago Press, 1924, chapter on Cytological Constituents.

14. Hyman, O. W.: Spermic Dimorphism in Fasciolaria Tulipa, J. Morphol. **37**:307, 1923.

15. Legendre, quoted by Cowdry (footnote 13).

16. Cajal, quoted by Cowdry (footnote 13).

similar to those found by Barratt and Arnold in their rats irradiated by roentgen rays. Hence it seems warranted to say that the effects of roentgen rays on germinal epithelium, as well as on the skin and subcutaneum, are probably due not to roentgen rays per se but to the electrons or cathode rays set free in the tissues.

These studies suggest that cathode rays applied to the abdominal wall of female rats might in a similar manner produce changes in the ova. Experiments with fertility should also be made for further comparison with the well known effects of irradiation with roentgen rays.

SUMMARY

A study was made of the effects of cathode rays of high voltage on the germinal epithelium of the male adult white rat. The shaved scrotal area was exposed in front of the anodal window of the Coolidge cathode ray tube at voltages of 200,000 and 250,000, with a current of 1 ma. The length of the exposure varied from five-tenths second to thirty seconds.

The changes in the scrotal skin were similar to those in the abdominal skin as described previously by Waddell and me.

Definite lesions were produced in the seminiferous tubules in a zone about 0.6 mm. in depth. The lesions consisted of degenerative and necrotic changes resembling those caused by roentgen rays. The tela subcutanea below the upper 0.2 mm. of the skin and the tunica vaginalis testis showed no evidence of injury, indicating clearly the much greater sensitivity of germinal epithelium to this form of irradiation. Alterations in the Golgi apparatus of the germinal cells were also observed, which correspond with the observations in the testis of *Cavia cobaya* irradiated with roentgen rays, as reported by Gatenby and Wigoder.

These observations are offered as further biologic evidence that the effects of roentgen rays are not due primarily to the roentgen rays but to the electrons or cathode rays set free in the tissues.

OSTEOGENESIS IMPERFECTA CONGENITA

A STUDY OF ITS HISTOPATHOGENESIS *

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The progress made in the histology of normal and pathologic bone within the last decade encourages a renewed investigation of the histopathogenesis of osteogenesis imperfecta. According to von Recklinghausen,[1] thirty cases of osteogenesis imperfecta (osteopsathyrosis congenita or fragilitas ossium congenita) were known in the literature to 1910. This number has been greatly increased within the last twenty years by the utilization of the roentgen rays in diagnosis. It is superfluous to review here the papers which may be found in the literature of the various countries, since that has been done recently by Dietrich.[2] The clinical and roentgenologic picture of osteogenesis imperfecta is well known. Therefore, a purely descriptive case report is no longer justified.

The cause of the disease is as obscure as the clinical and roentgenologic aspects are obvious. The theories concerning its cause (i. e., disturbances of internal secretion) are proved by neither morphologic nor biochemic observations. "The assumption of a pluriglandular deficiency," said Dietrich, "is a conception without foundation." Since the etiology is unknown, we have also no rational therapy.

Knowledge of the pathogenesis of the disease is intermediate to the well known clinical picture and the totally unknown cause. Only few definite facts have been added recently to the older knowledge of the histogenesis, since the classic work of von Recklinghausen. All authors have agreed that a lack of osteoblasts (a dysfunction, Bauer[3]) characterizes the histologic picture. But there is not a complete lack of bone, but rather a tissue of inferior quality. The question arises whether this inferiority must be traced to an original "vitium primae forma-

* Submitted for publication, Nov. 7, 1929.

* This study was aided by grants from the John C. and Edward Coleman Memorial Fund.

* From the George Williams Hooper Foundation for Medical Research, University of California.

1. Von Recklinghausen, F.: Untersuchungen über Rachitis und Osteomalacie, Jena, Gustav Fischer, 1910, p. 472.

2. Dietrich, A.: Die Entwicklungsstörungen des Knochens, in Henke-Lubarsch: Handbuch der speziellen Pathologie und pathologischen Anatomie, Berlin, Julius Springer, 1929, vol. 9, p. 198.

3. Bauer, K. H.: Ueber Osteogenesis imperfecta, Deutsche Ztschr. f. Chir. **154**:166, 1920.

tionis" (Kaufmann,[4] Dieterle[5] and Sumita[6]) or to disturbances which influence the bone-forming tissue secondarily. In any case, the lack of bone tissue, as well as the pathologic changes in the bone tissue actually present, is an expression of the underlying cause. The histologic features of the bony system represent an angle from which the problem must be tackled. Thus, one sets the question "How" before the question "Why."

In this deductive, analytic procedure, I shall be guided by the following considerations in regard to the complicated structure of bone, in general: Bone tissue is a local conglomeration of elements which represent the single time phases of the development of bone. Since knowledge of the normal is the foundation for the understanding of pathologic changes, the conglomerate of normal bone ("normal bone picture") must be separated into its time components. These components arranged according to their phylogenetic and ontogenetic appearances represent the "normal bone-building system," the general principles of which I have attempted to show in a previous paper.[7] Pathologic bone tissue represents a quantitative and qualitative caricature of the normal, and also a repetition—though modified—of the single stages of normal development. Thus, the single phases of the normal bone-building system must be balanced against those of the pathologic bone. Before this can be accomplished, the pathologic bone structure ("pathologic bone picture") itself must be divided into its time components.

Summarized, the question as to the pathogenesis of osteogenesis imperfecta may be stated as follows: "How have the time phases of the normal bone-building system changed quantitatively and qualitatively in order that the local conglomerate of the pathologic bone structure—as observed in osteogenesis imperfecta—might come about?"

The investigations were based on a case of osteogenesis imperfecta in a 2 day old girl recently observed, which will first be reported briefly.

REPORT OF CASE

History.—Both parents were strong and healthy with essentially negative family histories. It was their first child. The pregnancy was normal, the

4. Kaufmann, E.: Pathology, translated by Reimann, Philadelphia, P. Blackiston's Son & Company, 1929, vol. 2, p. 1176.

5. Dieterle, T.: Die Athyreosis, Virchows Arch. f. path. Anat. **184**:111, 1906.

6. Sumita, M.: Beiträge zur Lehre von der Chondrodystrophia foetalis (Kaufmann) und Osteogenesis imperfecta (Vrolik), Deutsche Ztschr. f. Chir. **107**:1, 1910.

7. Weber, M.: Bone Pathology and Its Relation to the Problem of Otosclerosis, Ann. Otol. Rhin. & Laryng. **37**:1232, 1928.

mother having medical supervision and direction in diet. The delivery was normal and at term. The child was weak and cyanotic. An abnormal skull and head were noted. Therefore, roentgen examination was made. The report of the roentgenologist, Dr. E. Schulze Heald, read as follows: "Osteogenesis imperfecta with many old and recent fractures of long bones and ribs. Very imperfect ossification of the skull." Each successive roentgenogram before and after death revealed more fractures. The cyanosis became greater, and the weakness more accentuated. Death probably was due to progressive asphyxiation. The body was preserved by the injection of formaldehyde through the left ventricle and by submergence in formaldehyde and was given to me for autopsy. (Dr. E. Schulze Heald, roentgenologist, Alta Bates Hospital, Berkeley, Calif., turned this case over to me for microscopic examination.)

Habitus.—The weight of the body was 5 pounds (2.3 Kg.). The soft parts were well developed. The subcutaneous fat was abundant. The extremities were extremely bowed, the lower extremities so much so that the plantar surfaces of the feet were approximate. The extremities were plump, but not malformed. They showed abnormal motility accompanied by marked crepitation. The neck appeared short. The appearance of the head suggested a bitemporal swelling. The growth of hair was conspicuously abundant. The skull felt like a rubber ball (Hildebrandt [8]). The capsule was thin, like parchment.

Organs.—Autopsy of the cavities of the body did not reveal any special abnormalities. The development of the organs was normal relative to the age, and there were no macroscopic pathologic changes. The following organs were examined microscopically: thymus, heart, lungs, liver, gallbladder, spleen suprarenal glands, kidneys, pancreas, uterus and ovaries. The examination of the specimens was made difficult by the aforementioned injection of formaldehyde. There was, however, a hyperemia of all organs and, besides, much hemosiderin, as was demonstrated by the Turnbull-blue reaction. Otherwise, the organs did not reveal any special microscopic changes.

General Observations in the Bone System.—The calvarium was easily dissected by means of scissors. The frontal and occipital bones were harder and from about 1 to 2 mm. thick, while the remaining part of the calvarium was almost purely membranous. Smaller and larger bony islands were embedded in this membranous capsule. Holding the calvarium to the light, these islands revealed an astral structure, like ice flowers. The space between the islands was translucent. Autopsy of the brain itself showed no macroscopic abnormalities.

The long bones were separated with great difficulty from the soft parts. The bones revealed many fractures, which may be seen well in the x-ray plate. One dealt here partially with fresh fractures (i. e., sixth and seventh left ribs and fifth right rib, left scapula, left humerus), partially with old fractures with more or less well developed callus formation (i. e., ninth and tenth left ribs, sixth, seventh, ninth and tenth right ribs, left radius, left ulna, right humerus and both femora). Several ribs were fractured twice. The fresh fractures were without doubt of postmortem origin; the older fractures evidently occurred in utero, as may be concluded from the large quantity of callus.

In general, longitudinal sections of the long bones presented an identical picture which differed only by the special features of the fractures. The conditions will be discussed, those in the right femur being used as an example (fig. 1). The isolated bone was elastic and flexible as rubber. There was no continuity of the cortex. Also a real spongiosa was not present. The bone may

8. Hildebrandt, H.: Ueber Osteogensis imperfecta, Virchows Arch. f. path. Anat. **158**:427, 1899.

be compared with a soft tube of periosteum (extending between the two epiphyses) which is studded internally with splinters of bone. The marrow was conspicuously red. In the line of fracture, there was a fissure, lined proximally and distally with white tissue which microscopically proved to be cartilage, thus indicating a pseudo-arthrosis. The proximal epiphyseal line was straight; the distal, gently curved. The cartilage of the distal epiphysis had an ossification center which was large, if one considers the age of the child. Let me emphasize that the incomplete formation of a cortex, as well as the straight osteochondral junction, repeated itself in all the long bones inclusive of the ribs, in the same manner.

THE DIAGNOSIS

In order to be able to diagnose osteogenesis imperfecta, according to von Recklinghausen [1] a triad of symptoms must be demonstrated:

1. "The fissures and fractures of the skeleton must have occurred repeatedly." In our case, this postulate was easily fulfilled. Numerous old and new fractures dominated the picture.

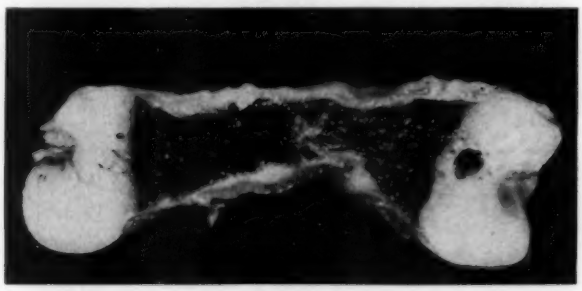

Fig. 1.—Longitudinal section through the right femur. Periosteal tube between the epiphyses, studded interiorly with bone splinters. Ossification centers in distal epiphysis. Straight epiphyseal line. Pseudo-arthrosis in the middle of the diaphysis.

2. "The fractures must have occurred in early life and must be traceable into the intra-uterine period." Since the child under discussion was only 2 days old, and many of the fractures revealed abundant solid callus formation, it may be concluded that this requirement of von Recklinghausen was also fulfilled.

3. "There must be such structural conditions of the bone as will account for the spontaneity of the fractures." It has been seen that the bone formation as a whole in the case under consideration was so imperfect that one could not speak of a spongiosa, and could speak of a cortex only in a limited sense. The calvarium was almost purely membranous, showing only discrete centers of ossification. The details of the bone structure will be treated in the following chapter. Without doubt, it will become still more evident that this factor was also present.

In brief, the diagnosis of the roentgenologist may be confirmed. One is dealing with a typical case of osteogenesis imperfecta.

THE HISTOLOGIC STRUCTURE OF THE BONE IN THIS CASE

The Osteochondral Junction.—As may be concluded from the examination of a great number of specimens all osteochondral junctions presented, in general, the same picture. I shall describe the conditions found in the fourth right rib, which were typical (fig. 2). The epiphyseal line was almost always straight. The columns were for the most part regular; occasionally, though, there were several columns in one block, surrounded by intercellular substance. The opening up of the cartilage always occurred in a normal manner. The intercellular substance between the columns showed a normal degree of calcification. Nowhere was there a superabundance of uncalcified cartilage as in the case of rickets, nor was

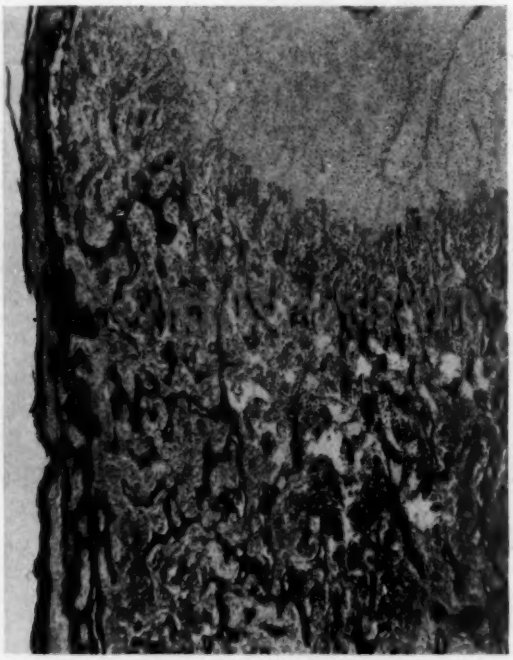

Fig. 2.—Osteochondral junction of the fourth right rib. No particular disturbance of the cartilage. Regular opening up. Free cartilage splinters, well calcified. No bone deposition on the same. Superabundance of lymphoid marrow. Specimen fixed in collodion; stained with hematoxylin-eosin. Leitz: Objective 1, eyepiece 4 x, extension 51 cm., magnification 26.1 : 1.

there a true rosary. The deposition of bony substance on the cartilaginous remnants was minimal or even completely lacking. As a result of this, there was no continuous framework of spongiosa. Rather, the cartilaginous remnants lay as free splinters in the marrow. Besides, they often lay at right angles to the long axis of the bone; i. e., they paralleled the epiphyseal line, as observed for instance in the right ulna (fig. 3). Wherever bone had been deposited on the cartilaginous remnants, it stained blue with hematoxylin and was evidently well calcified. There was no sign of a lamellar arrangement.

The cortex.—A transverse section through the long bone (e. g., the tibia) gave a good idea of the construction of the cortex. A cross-section of the tibia was decalcified for twenty-four hours in 5 per cent nitric acid, embedded in paraffin, cut 10 microns thick and stained with hemalum-eosin or Van Gieson's stain. Viewed under low power (fig. 4), it became evident that the cortex consisted only of a loose framework, thus resembling a spongiosa. Some of the splinters lay entirely free. In such areas, there was no bone separating the marrow from the periosteum. A conspicuously fibrous periosteum surrounded the framework. The marrow was present in much greater abundance than bone tissue. Viewed under higher magnification, the cortex showed the following details (fig. 5):

Fig. 3.—Proximal osteochondral junction of the left ulna. Crosswise position of the cartilage splinters in the lymphoid marrow. Minimal fiber bone formation under the periosteum. Specimen fixed in collodion; stained with hematoxylin-eosin. Leitz: Objective 1, eyepiece 4 x, ext. 50 cm., magnification 25.6:1.

Toward the lumen, there was a marrow consisting of lymphoid elements. Toward the periphery were situated islands of peculiar bone tissue. These islands, surrounded by a loose fibrous tissue, showed a dark blue marginal zone after hematoxylin-eosin staining. The inner area of the islands appeared lighter. Occasional darker spots, however, could be observed, as well as granular crumbly regions, which occur not infrequently in cases of irregular and pathologic calcification. Often the entire bone structure of the cortex was fragmentary and scaly (fig. 6). The bone cells were monstrous and plump. After being stained with thionin-picric acid, these cells showed only thick and short canaliculi. Besides, they lay irregularly. A lamellar arrangement could not be observed.

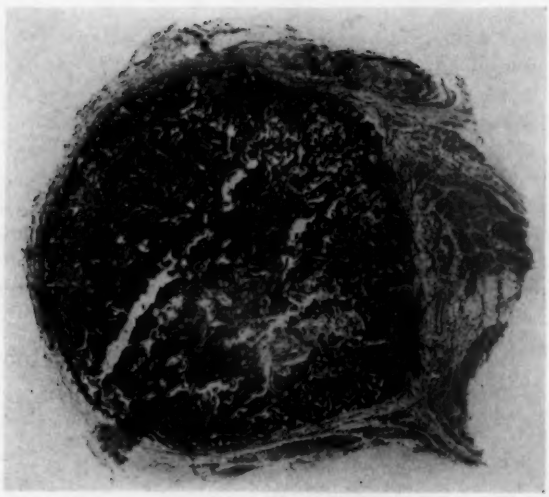

Fig. 4.—Transverse section of the diaphysis of the right tibia. Lack of spongiosa. Loose cortex consisting of splinters of fiber bone surrounded by a dense fibrous tube of periosteum. Gelatin. Frozen section. Hemalum-eosin. Leitz: Summar 80, ext. 77.5 cm., magnification 9:1.

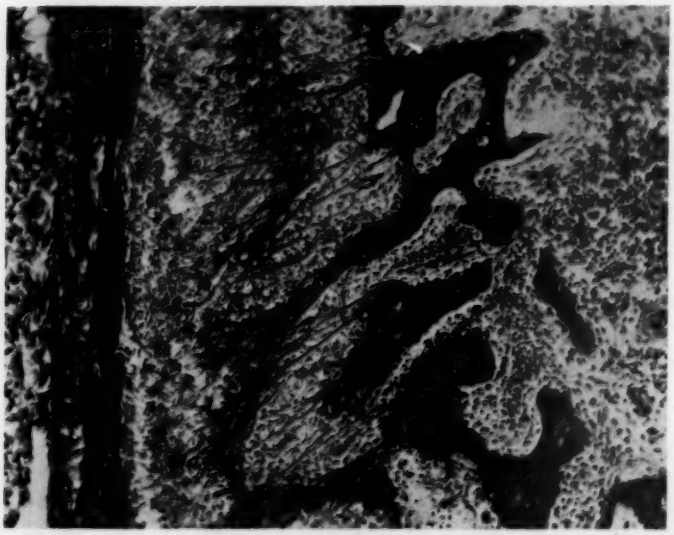

Fig. 5.—The same specimen as shown in figure 4, under higher power. Internally, lymphoid marrow. More peripherally, calcified fiber bone surrounded by reticulated and fibrous marrow. Under the periosteum, islands of fibroblastic osteoid surrounded by fibrous marrow. Toward the upper margin of the picture, dense periosteum. Parowax. Iron-hematoxylin, van Gieson's stain. Leitz: Objective apochromate 16 mm., eyepiece 4 x, ext. 40 cm., magnification 76.8:1.

After staining with van Gieson's stain, one observed that fibers crossed each other within the islands. One dealt with a coarse fiber bone.

Toward the marrow cavity, this fiber bone was well calcified, though irregularly. Toward the periosteum, a younger stage of fiber bone was observed in which the calcification was still, in part, completely lacking. This young bone tissue appeared at first in the form of islands, which were connected by coarse fibers, on the one hand, with the cambium layer of the periosteum and on the other hand, with the older, already calcified, fiber bone. These young stages gave an excellent opportunity for studying the modus of fiber bone formation (fig. 7). The intercellular substance increased between the cells, without the aid of osteoblasts arranged in an epithelial row. The cells enclosed by the inter-

Fig. 6.—Same specimen as shown in figures 4 and 5. Fiber bone of the cortex under high power. Scaly and granular-crumbly appearance of the intercellular substance. Large vesicular cells. No active endosteum in the vicinity. Paro-wax. Hemalum-eosin. Leitz: Objective 6 a, eyepiece 4 x, ext. 51 cm., magnification 357 : 1.

cellular substance remained lying in their original position and seemed passive. Their morphologic appearance did not change, except for a slight rounding off of the outline of the cell. The intercellular substance stained red with eosin. The islands must be regarded as "fibroblastic osteoid," which is the preliminary stage in the formation of fiber bone.

A detailed examination of all important bones of the skeleton revealed that the entire bone system was formed by such fiber bone. It was not normal, however, but rather pathologic in its appearance, probably as a result of changes in the intercellular substance, as indicated by the blue color after hematoxylin-eosin staining.

Summarizing, one concludes that in this case the whole bone system consisted of pathologic fiber bone, which partially was still in a relatively low stage of development.

The Fractures.—The fresh fractures had little interest for these investigations. Hemorrhagic foci, inclined to organization and hyaline transformation, had arisen between the fragments. The designation "fragment" could be used often only in a limited sense, since not always a real fracture had occurred. Indeed, the bone tissue was in some places so lacking in continuity that even without a "fracture," the slightest movement (Bauer,[3] Harbitz,[9] von Recklinghausen [1]) might produce a hemorrhage with all its consequences. The majority of these so-called fractures were in reality "pseudofractures."

The older fractures and pseudofractures already showed reactions of the bone-building system. A good example for studying these changes was offered by

Fig. 7.—Same specimen as shown in figure 4. Beginning of fiber bone formation, fibroblastic osteoid. Inclusion of the cells of the fibrous tissue in situ by increase of intercellular substance, later calcifying. No osteoblasts in rows. Collodion. Iron-hematoxylin, van Gieson's stain. Leitz: Objective 6 a, eyepiece 4 x, ext. 40 cm., magnification 280: 1.

the fractures of the left forearm, in which the x-rays (fig. 8) indicated an enormous callus formation. Viewing with middle power, one observed that a new formation of cartilage and bone had occurred in the region of the fracture (fig. 9). The cartilage islands were easily distinguished by their light color, after being stained with thionin-picric acid (fig. 10). These cartilaginous portions had evidently originated from the periosteum, as well as from the marrow cavity. Distal to these cartilaginous new formations there was a fissure, which was limited by new-formed bone.

9. Harbitz, F.: Ueber Osteogenesis imperfecta, Beitr. z. path. Anat. u. z. allg. Path. **30**:605, 1901.

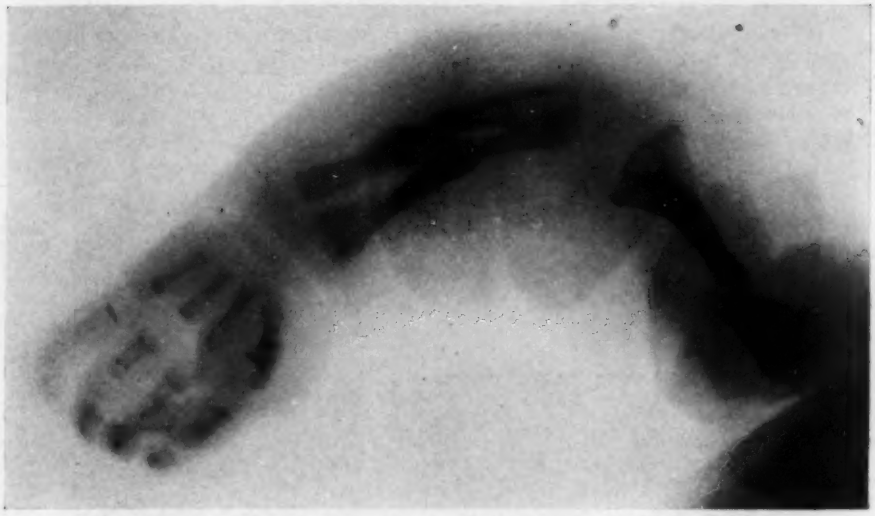

Fig. 8.—Roentgen picture of the left arm showing fresh fracture of the humerus and old fractures of the radius and ulna with abundant callus (x-ray picture supplied by courtesy of Dr. E. Schulze Heald).

Fig. 9.—Longitudinal section of the left ulna. Callus formation. New formation of cartilage and fiber bone. Collodion. Hematoxylin-eosin. Leitz: Objective 1, eyepiece 4 x. ext. 50 cm., magnification 25.6 : 1.

The exâmination of the detailed development and structure of the cartilage showed that the cartilage had arisen, after the manner of the fiber bone, from the loose mesenchymal tissue of the periosteum and marrow. Here, also, the intercellular substance played an important rôle (chondroid). But the cells, too, had undergone changes, from a spindle cell appearance to a more round and vesicular form. Aside from this, there were certain transitional areas in which neither the form of the cells nor the intercellular substance revealed whether one is dealing with osteoid or chondroid.

The new formation of bone tissue was increased in the neighborhood of the fractures (fig. 10). There was a remarkable thickening of the cortex, whereby the real callus was produced. However, this cortex, too, was loose. It continued, centripetally, to a spongiosa which was conspicuously dense. Thus, there

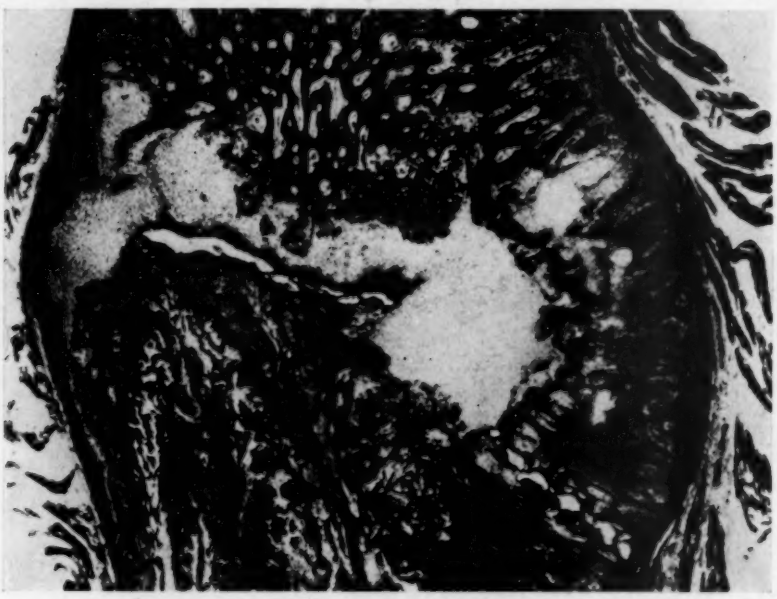

Fig. 10.—Longitudinal section of the left radius. Abundant formation of cartilage in region proximal to the fracture (light color). Also intensive new formation of fiber bone. Fissure, a probable pseudo-arthrosis. Collodion. Thionin-picric acid (Schmorl). Leitz: Objective 1, eyepiece 4 x, ext. 50 cm., magnification 25.6: 1.

was no structural difference between the loose cortex and the dense spongiosa. The whole bone tissue surrounding the fracture, also, consisted of fibroblastic bone (fiber bone). Only at one point, an island of fiber bone was covered with one or two layers of lamellar bone, lined with osteoblasts in epithelial arrangement. This margin stood out clearly after the section had been stained with thionin-picric acid. Its bone cells were arranged neatly, paralleling the lamellae, and showed graceful dendritic canaliculi. The remaining portion of the callus showed the same absence of lamellar margins as did the skeleton in general. Where osteoblasts in epithelial rows covered the bone, there were a few discrete giant cells which resorbed the new-formed cartilage, as well as the bone, by

lacunar resorption. Let me here emphasize that the callus tissue was the only place in which osteoblasts in rows and osteoclasts could be observed. Neither in the petrous bone nor in the vertebrae could a single osteoclast be found.

The Marrow.—There were two types of marrow, a lymphoid and a fibrous (fig. 5). The incidence of these two forms was limited to definite areas; in general, the lymphoid marrow predominated.

The lymphoid marrow filled the entire marrow cavity of the bone and the center of the spaces between the splinters of the cortex. There was a great variety of cells. Besides lymphoid and myeloid elements there was a number of eosinophils and nucleated reds (fig. 11). Hemorrhages also were observed.

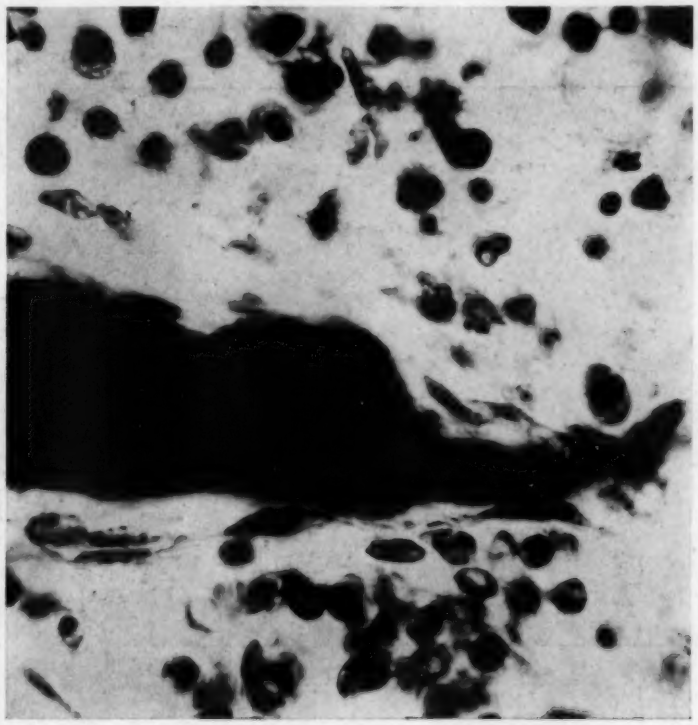

Fig. 11.—Bone splinters lying in the marrow cavity, surrounded by long spindle cells. Various types of cells in the marrow: myeloid and lymphoid elements, eosinophils and nucleated reds. Parowax. Hematoxylin-eosin. Leitz: Objective oil immersion apochromate 3 mm., eyepiece 4 x, ext. 85 cm., magnification 918: 1.

In some cases, this marrow reached directly to the bone splinters, which took the place of true spongiosa. In the osteochondral junction, also, this marrow continued to the epiphyseal line.

The fibrous marrow was observed (1) in the centers of the loosened cortex (to the extent that they were not filled in the center with lymphoid marrow), as illustrated in figure 5; (2) toward the periosteum (fig. 7), and (3) within the great marrow cavity directly surrounding the bone splinters (fig. 11). This

marrow resembled young mesenchymal tissue with its astral mother cells, which are connected by their extremely delicate fibrils. Lymphoid elements were disseminated throughout this tissue. Toward the great marrow cavity, this tissue gradually continued into the reticulum of the latter. While the fiber marrow became loosened toward the lymphoid marrow spaces, it became more dense toward the bone. Especially dense and purely fibrous was the tissue underneath the periosteum and within the marrow cavity of the callus. At those places where fibroblastic bone formation occurred, one might observe that these cells became more rounded (fig. 12). An intercellular substance appeared, in which the fine fibers became embedded. From here, the fibers radiated into the reticulum not yet condensed. When a fibroblastic osteoid had appeared, it remained surrounded by a loose network of spindle cells (fig. 11). Also, the cartilaginous remnants were covered with such loose fibrous tissue.

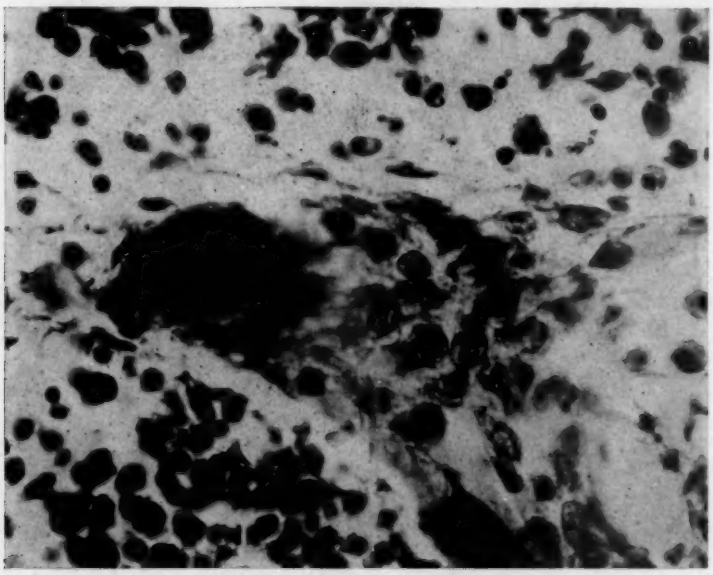

Fig. 12.—Fibroblastic bone formation in the marrow cavity. Rounding off of the astral mother cells of the reticulum. Increase of the intercellular substance. No osteoblasts in rows. Parowax. Hematoxylin-eosin. Leitz: Objective 6 a, eyepiece 4 x, ext. 85 cm., magnification 597 : 1.

THE PRINCIPLES OF THE NORMAL BONE-BUILDING SYSTEM

The knowledge of the single phases of the normal bone-building system is the foundation for the conception of the bone changes observed in osteogenesis imperfecta. Therefore, these phases of normal bone development will be discussed briefly. I presented them in detail about a year ago.[7] In accordance with newer knowledge, one must recognize five stages, each distinguishable by the individuality of its morphologic picture.

1. *The Undifferentiated Mesenchymal Tissue.*—The mesenchyme, after its development from the omnipotent germ, possesses only a pluripotent character. It is represented, as far as bone is concerned, by a loose reticulum, the so-called "reticulated marrow." The mother cells have an astral form and are connected by fine fibrils. This stage is the fundamental phase of the bone-building system.

2. *The Differentiation and Increase of the Intercellular Substance.*—The mesenchymal tissue differentiates in two directions. On the one hand, a fibrous tissue (fibrous marrow) originates containing long spindle cells, which soon are embedded in a homogeneous, hyaline matrix, staining red with eosin. The cells of the fibrous tissue are enclosed in situ, whereby they are slightly forced apart and rounded up. They lie irregularly in the increased intercellular substance. This stage is preliminary to the formation of fiber bone; one is dealing with "fibroblastic osteoid." On the other hand, more vesicular cells differentiate from the mesenchymal tissue, which are embedded in intercellular substance staining reddish blue. One is dealing here with "chondroid," the precursor of cartilage formation. If the formation of cartilage is completed, the intercellular substance stains almost pure blue with hematoxylin-eosin. It is plausible that, under pathologic conditions, a transition (so-called metaplasia) of one of these preparatory stages into another takes place by changes in the composition of the intercellular substance. Normally, this tissue has already unipotent character, which manifests itself in the development of the next stage.

3. *The "Nucleus" Formation.*—The two intercellular substances of the preliminary stage become calcified. Both products represent the foundation for the later deposition of lamellar bone. Thus, two "nuclei" originate: (1) the "connective tissue nucleus" or "fibroblastic nucleus," i. e., the fiber bone, and (2) the "cartilaginous nucleus," i. e., the calcified cartilage. The structure of cartilage is generally known. It represents the model for the form of the most quickly growing bones. In principle, the structure of fiber bone is as follows: Its intercellular substance stains red or bluish red. The bone cells lie irregularly. They are plump and have short, thick canaliculi, which can be demonstrated only with difficulty by the thionin-picric acid method. This fiber bone is phylogenetically and ontogenetically the oldest type of bone. In general, there are three places where fiber bone may be observed: (1) as the "nucleus" of the bones preformed in membrane; (2) presumably as the first deposition of bone under the periosteum, and (3) invariably in the tendinous insertions.

4. *The Shell Bone Formation.*—The lamellar bone (shell bone) is deposited on the previously formed "nuclei." In this process, cells participate which are arranged in an epithelial row. These osteoblasts

stand in close relation to the blood vessel system. Presumably, they originate from the endothelial cells of the blood vessels. These cells rise on edge to a vertical position, with relation to the bone. They have a basophilic plasma, and their nuclei lie basally and show no definite chromatin structure. Before the lamellar bone is deposited, the "nucleus," previously formed, becomes vascularized in many cases. This vascularization is well known, especially in regard to the "cartilaginous nucleus." Thus, the cartilaginous model is hollowed out. The "fibroblastic nucleus," however, from the start, has enclosed blood vessels, which makes a separate process of hollowing out unnecessary. These conditions may be well recognized in the calvarium. The lamellar bone deposited around the vessels forms the haversian system, the osteon. This represents the ontogenetically and phylogenetically highest unit of bone development. The cells of this shell bone are long and slender. The delicate canaliculi may be demonstrated best by the thionin-picric acid method (Schmorl[10]) or with ground sections (Weber[11]). The cells lie in regular order. The fact that connective tissue fibers (Sharpey fibers) are included in this lamellar bone under the periosteum offers no hindrance to the classification of this bone (lamellar fiber bone, Kölliker) with the shell bone of Weidenreich. This conception is based on the observation that the principle modus of the development of the so-called lamellar fiber bone (except for the fact that it contains fibers, owing merely to its location) is identical with that of the true lamellar bone of the endosteum, which normally never contains fibers.

5. *The Resorption.*—Each of the foregoing processes may be interrupted at any point by processes of resorption. Most frequently, the resorption involves the highest unit, the shell bone. Thus, the resorption must be regarded as a separate stage, in which the already completed bone is carved into its ultimate form. The resorption is closely related to the blood vessel system. The blood vessels themselves may have a resorbing influence by means of their walls. For the most part, the resorption is induced by osteoclasts. They are supposed to have originated as "detached vessel buds" from the endothelial cells of the blood vessels. Each may have one or more nuclei. They represent the same tissue cells as the osteoblasts, only in a different functional rôle. In normal bone there is a definite correlation between the function of the osteoblasts and that of the osteoclasts.

10. Schmorl, G.: Die pathologisch-histologischen Untersuchungsmethoden, Leipzig, F. C. W. Vogel, 1925, p. 259.

11. Weber, M.: Schliffe von macerierten Röhrenknochen und ihre Bedeutung für die Unterscheidung der Syphilis und Osteomyelitis von der Osteodystrophia fibrosa sowie für die Untersuchung fraglich syphilitischer, prähistorischer Knochen, Beitr. z. path. Anat. u. z. allg. Path. **78**:441, 1927.

Disturbances in this correlation are pathologic and lead to changes in the form of the bone.

The description of the system of the bone-building apparatus just given is a schematic presentation of morphologic observations. Therefore, it has its weak points just as any schema of biologic processes. The difficulties may in the future be eliminated by further investigations on the basis of the facts already gained. At present, however, as a working hypothesis, this scheme is of the greatest significance in investigations of the nature of bone diseases as yet obscure. In a recent detailed German paper,[12] I attempted to analyze the nature of osteodystrophia fibrosa (so-called ostitis fibrosa) on the basis of this schema. Thus, the nature of this disease could be explained in an entirely new manner. The observations did not contradict the older knowledge, but rather supplemented it.

Based on the typical case of osteogenesis imperfecta (reported herein), the following chapter will be devoted to the analysis of the nature of osteogenesis imperfecta.

DEVIATIONS OF THE BONE-BUILDING SYSTEM FROM THE NORMAL IN OSTEOGENESIS IMPERFECTA

The Undifferentiated Mesenchymal Tissue.—Qualitative changes of this stage could not be recognized morphologically. A loose reticulated marrow with disseminated lymphoid and myeloid elements occupied the cavities of the cortex and surrounded the cartilaginous remnants situated within the marrow cavity. A great number of these splinters lay completely or almost entirely free, without surrounding reticulated marrow (fig. 6). Von Recklinghausen already has observed this lack of "germinal tissue." He found a superabundance of lymphoid marrow at the expense of bone-forming tissue. He believed it to be a "metaplasia," a transformation of bone-forming into blood-forming tissue (or marrow). Therefore, he designated osteogenesis imperfecta as "myeloplastic" malacia. While the tissue of the first stage was qualitatively normal, as to morphology, it is in its entirety quantitatively reduced.

The Differentiation and Increase of Intercellular Substance.—The preliminary stage in the development of fiber bone could be demonstrated clearly in each of the bones examined. Particularly under the periosteum could differentiation of mesenchymal cells into fibrous tissue be observed in which an increase of intercellular substance appeared (fig.

12. Weber, M.: Osteodystrophia fibrosa, ihre pseudotumor- und cystenbildende, juvenile, hyperostotisch-porotische Form beim Hund, zugleich ein Beitrag zur Frage ihrer Pathogenese und Aetiologie, Beitr. z. path. Anat. u. z. allg. Path. **81**:383, 1929.

7), repeating the normal process. This preliminary stage of fiber bone formation (fibroblastic osteoid) was found also in the callus of the fractures. Here, the marrow spaces were nearly completely filled with fibrous marrow.

The cartilage tissue appeared normal (Schmidt,[13] Dietrich,[2] Harbitz [9]), relative to the age of the child. In several bones, larger cartilage islands were located below the periosteum midway between the epiphyseal line and the middle of the diaphysis. In the region of the fractures, a cartilage formation occurred in a so-called "metaplastic" manner. This cartilage, however, did not originate in a truly metaplastic way, but rather originated from a proliferation of a germinal tissue ontogenetically destined to provide either cartilage or fiber bone. The stimulus for the local proliferation of cartilage is probably referable to mechanical factors.

As far as morphology was concerned, there were no special disturbances of the preliminary phase, qualitatively. In comparison with that in a normal child of the same age, this stage was also not reduced quantitatively. Nevertheless, in the region of the fractures (i. e., under abnormal conditions) an increase of this stage was evident.

The "Nucleus" Formation.—The cartilage was regularly opened up (Bauer [3] and others) by means of blood vessels. The intercellular substance was calcified in a normal degree. The cartilage, also, underneath the periosteum and the callus showed a normal degree of calcification. Aside from the conditions prevailing in the fractures, the cartilaginous "nucleus" formation was not increased quantitatively and was qualitatively of normal morphology.

In regard to the fibroblastic bone formation, conditions were markedly different. In comparison with a normal new-born infant, the following is stated: Normally, fiber bone is found in great abundance between the osteons of the cortex, as a so-called "rootstock" (Gegenbaur) and invariably at tendinous insertions. Imagine for a moment that these haversian systems have disappeared. A skeleton of fiber bone remains. If now, one compares this hypothetical normal framework of fiber bone with the fiber bone actually present in osteogenesis imperfecta, one notices that there is no smaller quantity of fiber bone than in the normal state. Indeed, there seems to be more fiber bone in osteogenesis imperfecta than in the normal state. This may be explained in two ways: On the one hand, more fiber bone may have been formed (i. e., absolute increase) or, on the other hand, the fiber bone, though formed in normal quantity, was not sufficiently resorbed (i. e., relative increase). Probably, the latter offers the more satisfactory explanation, for no

13. Schmidt, M. B.: Der Bewegungsapparat, in Aschoff: Pathologische Anatomie, Jena, Gustav Fischer, 1928, vol. 2, p. 190.

process of resorption by osteoclasts could be observed, except in the regions of the fractures. The thickening of the bone in the callus was due to abundant fiber bone. Thus, an absolute increase of fiber bone was indicated in this region. This fiber bone, however, did not exceed a callus formation which might be observed in any callus of a normal bone.

Now the question of the quality of the fiber bone arises. It was stated that there were severe changes in the composition of the intracellular substance, which appeared now scaly, now granular-crumbly or with bluish-red stippling. A great number of splinters had a deep blue margin, which von Recklinghausen [1] interpreted partly by "trypsis." On the whole, the calcification seemed to be quantitatively normal, for the newly formed fiber bone stained conspicuously blue. This observation has also been made by nearly all earlier authors. There was evidently a disturbance in the composition of the intercellular substance, which was of inferior quality. The cells included in the intercellular substance also showed changes, which may be related to the changes in the matrix. The cells were peculiarly round and vesicular. Their nuclei were rounded, too, and had a clearly visible chromatin network. It does not seem plausible that these changes were caused by "oncosis" of formerly normal bone cells. Rather, this was a young newly formed fiber bone which was of inferior value from the start.

The Shell Bone.—Shell bone had originated only in a few layers in the neighborhood of the fractures. This lamellar bone did not appear as osteoid, but was immediately well calcified. Except for these few layers of lamellar bone, every trace of a shell bone formation was lacking in the whole skeleton (Bauer,[3] Looser [14]). The entire bone picture, then, exclusive of the fractures, gave the impression that the lamellar bone had been lost from the young bone of the new-born infant, and that only the framework of the rootstock, the fiber bone, had remained. An analysis of the bone picture reveals, however, that the lack of shell bone was under no circumstances referable to a direct disappearance (i. e., resorption) of shell bone formerly present, but rather to a complete lack from the beginning.

The Resorption.—Processes of resorption induced by giant cells were observed only in the region of the callus, where they were considerably augmented. Nowhere else in the whole skeleton could a single osteoclast be found, nor was any particular resorption by vessels to be demonstrated.

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14. Looser, E.: Zur Kenntnis der Osteogenesis imperfecta congenita und tarda (sogenannte idiopathische Osteopathyrosis), Mitt. a. d. Grenzgeb. d. Med. u. Chir. **15**:161, 1906.

THE NATURE OF OSTEOGENESIS IMPERFECTA

In discussing the analysis of the bone picture, the observations concerning the fractures will be disregarded temporarily. Their immediate consideration would complicate the conclusions at this stage. Although the fractures are an integral part of the clinical picture of osteogenesis imperfecta, they have arisen only secondarily. They must be regarded as a mechanically determined logical consequence of a primary disturbance of the bone system. The bone picture of the fractures in particular can be understood only if the nature of the osteogenesis imperfecta has been determined in general.

From the analysis of the bone picture, one may draw the following conclusions as to the nature of osteogenesis imperfecta.

1. Osteogenesis imperfecta is a generalized disease of the bone-building system. The entire skeleton is involved in the same manner.

2. The disease is congenital. Its origin is traceable far back into the intra-uterine period, as may be concluded from the dense callus formation surrounding the fracture.

3. The bone picture of osteogenesis imperfecta is one of arrested development which manifests itself both in a passive and in an active aspect.

4. The passive expression of the arrested development is the lack of the highest unit, the osteon. Either there are no osteoblasts present or they cannot perform their destined function of producing shell bone.

5. This arrest is not secondary, as in osteodystrophia fibrosa, but rather primary; i. e., a formation of lamellar bone has never occurred.

6. The active aspect of the arrested development finds its expression in the bone tissue actually present. The analysis shows that the entire skeleton in osteogenesis imperfecta consists of fiber bone. As stated, this type of bone is ontogenetically and phylogenetically the oldest type of bone tissue and forms the "nucleus" for the consequent deposition of shell bone. Thus, one may conclude that the bone tissue has been arrested in the stage of "nucleus" formation. Also cartilage is present as "cartilaginous nucleus."

7. The bone picture as a whole is a representation of the combined passive and active components of the arrested development. The result of the combination in regard to the bone picture is "a relative displacement to the left." This statement may require some explanation: The term "displacement to the left" is well known in connection with diseases of the blood-building system. If one should graphically present the quantity of the single stages of the bone-building system in osteogenesis imperfecta beginning from the left, the following would result: The highest stage of the development, on the extreme right, would be

completely lacking. The middle stages would be seemingly greatly increased, whereas they are in truth only relatively increased, i. e., the fiber bone is formed in normal quantity, but not resorbed by osteoclasts, which are lacking completely. The youngest stage would, in fact, be decreased absolutely.

8. The fiber bone forming the skeleton in osteogenesis imperfecta shows pathologic changes of its intercellular substance. One is dealing with a fiber bone of inferior quality. Since this pathologic fiber bone has arisen from an embryonal tissue of fetal origin one may well speak of a congenital faculty differentiation or malformation.

Summarizing: Osteogenesis imperfecta is a congenital primary generalized arrested malformation of the bone-building system with a "relative displacement to the left" of the bone picture.

In this connection, one may consider the relation to several other bone diseases. I have designated the nature of another of the "malacic" bone diseases, osteodystrophia fibrosa, as "irritation of the bone-building system with consequent (absolute) displacement to the left of the bone picture." Here, too, one observes malformations, which, however, originate from a differentiation of a newly arisen mesenchyme. This disease involves a formerly normal skeleton, and thus presents a secondary dystrophy, which may also appear in a localized form. In fact, in those places in osteogenesis imperfecta in which the arrest of differentiation has been replaced by a stimulation, the picture is identical with one of an osteodystrophia fibrosa. Such conditions are found in the regions of the fractures. Here all the requirements are met which von Recklinghausen postulated for the diagnosis of a typical "ostitis fibrosa": fibrous marrow, giant cells, fibroblastic osteoid and fiber bone formation. Cysts, also, were observed, and malformed fiber bone as actually observed in the pseudotumors or brown tumors in osteodystrophia fibrosa. By the stimulation of the bone-building apparatus in these areas, a minimal formation of shell bone has occurred in a manner like that observed in osteodystrophia fibrosa.

Another disease that is frequently mentioned in connection with osteogenesis imperfecta is otosclerosis. In a recent paper,[15] I designated otosclerosis as a localized osteodystrophia fibrosa. It is my intention to present in the near future the pathologic changes of the petrous bone in the present case and to investigate its relation to otosclerosis.

The relation of osteogenesis imperfecta to Moeller-Barlow's disease requires a brief consideration. Juvenile scurvy is characterized by the fact that shell bone apposition ceases, while normal resorption continues. This arrest of shell bone formation attacks an originally

15. Weber, M.: Otosclerosis in Its Histogenic Relations to Osteodystrophia Fibrosa (Ostitis Fibrosa), Arch. Otolaryng. **11**:1 (Jan.) 1930.

normal skeleton. Further investigations seem desirable in order to clarify the conditions as to the fiber bone. Besides, a hemorrhagic diathesis leading to hemorrhages is typical of scurvy. In my case, hemorrhages could not be observed within the organs, but were observed underneath the periosteum and within the bone-marrow. Presumably, the hemorrhages in osteogenesis imperfecta may be due to mechanical disintegration. From this it may be, perhaps, concluded, with all conservatism, that there are genetic relations between osteogenesis imperfecta and Moeller-Barlow's disease. Further research must clarify this question.

CONCLUSIONS CONCERNING THE CAUSE AND THERAPY

From the knowledge of the nature of osteogenesis imperfecta acquired from the analysis of its bone picture, one may make certain conclusions concerning the cause and therapy.

The etiology of osteogenesis imperfecta is still unknown. The question arises: "Is there a germinal (vitium primae formationis) or a secondary disturbance?" In order to clarify this question it seems advisable to investigate at first where the functional point of attack might be situated which could explain a germinal as well as a secondary disturbance.

In osteogenesis imperfecta, either the osteoblasts, as well as the giant cells, are lacking or they do not carry on their destined function. Both types of cells originate from the endothelial cells of the blood vessels. Thus, the endothelial cells in general may be held responsible for the changes in the bone picture. Severe arteriosclerotic changes observed by many authors in young children with osteogenesis imperfecta point likewise to an involvement of the walls of blood vessels.

This juvenile arteriosclerosis in cases of osteogenesis imperfecta indicates also a disturbance in the composition of the intercellular substance in the same sense as the pathologic fiber bone formation in osteogenesis imperfecta might indicate such a disturbance. Thus, one may conclude that the changes are traceable as far back as the mesenchyme (Bauer [3]).

Attempts have been made to solve the question as to whether one is dealing with a primary or a secondary disturbance of mesenchyme by examination of the teeth. It is plausible that the oldest hard substance of the body, the teeth, in osteogenesis imperfecta might be normal in their original anlage. Indeed, Biebl [16] stated that the primary layers of dentine observed in a case of osteogenesis imperfecta were normal. The teeth in my case will be examined by Dr. Hermann Becks, and the report will be published in the *Journal of the American Dental Asso-*

16. Biebl, M.: Beitrag zur Frage der Osteogenesis imperfecta durch Untersuchungen am Zahnsystem, Virchows Arch. f. path. Anat. **255**:54, 1925.

ciation. Dr. Becks has accorded me the privilege of giving out the following statement concerning the teeth: "The dentine in the case under discussion shows severe changes. There is, however, no indication that the dentine first deposited was ever normal." Thus, we cannot confirm Biebl in regard to this case and rather would like to agree with Bauer [3] and Kaufmann,[4] who believed that there is a vitium primae formationis. But, in spite of the observations, it might be possible that one is dealing with a secondary process; for, in the bone and in the tooth system identical histologic changes might be caused by a disturbance of the anlage or by an early secondary disturbance of the mesenchyme which persists. On the other hand, however, there is the improbable possibility that two types of osteogenesis imperfecta exist. The one would be a vitium primae formationis and the other a dystrophy, beginning early. An elucidation of this question could hardly be established by morphologic studies, but must be approached rather by experiments on pregnant animals. A diet (perhaps a modified scurvy diet) resulting in an arrested malformation of bone, producing a displacement to the left of the bone picture, may possibly produce an osteogenesis imperfecta in the offspring.

Also, in regard to therapy, one may develop some fundamental thoughts: The attempt must be made to overcome the arrest in development of the bone-building system which has its functional location in the walls of the blood vessels and the composition of the intercellular substance. The formation of the shell bone must be stimulated. Then, the loose framework of the cortex, consisting of fiber bone, would be filled with lamellar bone. How this might be accomplished no one exactly knows. Systematic investigations as to the influence of diets and other factors on the single phases of the bone-building system will have to clear these questions.

SUMMARY

The pathogenesis of osteogenesis imperfecta is examined from a new standpoint. The investigations are based, on the one hand, on a case of osteogenesis imperfecta observed in a 2 day old girl and, on the other hand, on the newer knowledge gathered from the international literature. The normal bone-building system, described previously by the author, is used as a foundation for the analysis of the observations. This system presents the single time phases of the normal development of bone, the combination of which is designated as the "bone picture." The deviation of the single phases from the normal constitutes the bone picture in osteogenesis imperfecta. It is demonstrated that the youngest stage in the case reported was qualitatively of normal morphology and quantitatively reduced. The highest stage, the lamellar bone or shell bone, was completely lacking, as was also the process of resorption by

osteoclasts. The conclusion is drawn that in osteogenesis imperfecta the entire bone system has been arrested in the stage of "nucleus formation," i. e., the bone actually present consists of fiber bone which, forming the foundation of lamellar bone, must be sharply differentiated morphologically from the latter. This fiber bone is of inferior quality and shows a relative increase in quantity. The cartilage is evidently normal and opened up in a normal way. Thus, osteogenesis imperfecta must be regarded as a congenital primary generalized arrested malformation of the bone-building system which leads to a "relative displacement to the left" of the bone picture. Presumably, the disease is traceable as far back as the endothelial cells of the blood vessels and the composition of the intercellular substance. Whether a vitium primae formationis or a secondary disturbance must be held responsible could not be determined in this case. The fractures are only a local secondary mechanical result of the primary disturbance of the bone system in general. They reveal the picture of osteodystrophia fibrosa, which the author has designated previously as "irritation of the bone-building system leading to an absolute displacement to the left of the bone picture." Finally, certain new points of view as to etiology and therapy are briefly discussed and methods suggested for the solution of questions still open.

ADENOFIBROSARCOMA OF THE BREAST*

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Sarcoma of the breast is in contrast with carcinoma of the breast in that while the latter forms about the best understood group of neoplasms, the former remains poorly classified, and the relation between pathologic type and clinical course is still obscure.

The confusion in the group of sarcoma of the breast has arisen from ill adapted criteria for its subdivision and the inclusion of all malignant tumors not definitely epithelial. On more painstaking examination, some of these tumors made up of large spindle-shaped cells loosely connected and without definite stroma have shown scattered foci of well-defined medullary epithelial structure (Ewing[1]). It is probable that most of the tumors described as carcinosarcoma and many of the round and spindle-cell alveolar sarcomas are really atypical carcinomas.

It is conceivable that in the connective tissue of the breast any tumor of mesenchymal origin may arise, as well as mixed tumors and teratomas. Such cases have been reported. Chondrosarcoma and mixtures of chondromatous and epithelial elements appear to be only moderately rare. In carcinoma, the stroma may undergo metaplasia and the occurrence of cartilage and bone in it has been reported.

One type of sarcoma appears to be specific for the breast; this is one in which an admixture of epithelium-lined spaces is found. In its earliest stage this type of tumor is identical with adenofibroma, except for a more active proliferation of the fibroblastic tissue. The relative importance of the epithelium and of the fibrous tissue in adenofibroma remains in some dispute. Certain authors (Virchow[2]) believe that the proliferation is primarily of the connective tissue, while others (Schimmelbusch[3]) consider that the fundamental and primary change is in the epithelium. At puberty and in pregnancy there is an active proliferation of acini accompanied by a proliferation of the pericanalicular and periacinous connective tissue, and such changes, localized and accentuated, perhaps in a superfluous or misplaced lobule, give rise to a

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1. Ewing, James: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1928, p. 540.

2. Virchow, R. L.: Die Krankhaften Geschwülste, 1863, vol. 1, p. 328.

3. Schimmelbusch, in Langenbeck: Arch. f. Chir. **44**:102, 1892.

mixed tumor, adenofibroma (Cheatle [4]). It would seem that there is a fairly even balance between the proliferation of the connective tissue and of the epithelium. Cases of pure fibroma of the breast (Williams [5]) and of pure adenoma of the breast (Cheatle [4]) are reported, but are extremely rare compared with the cases of mixed tumor.

Eventually, either the epithelial or the fibrous element may predominate in an adenofibroma, and from such overgrowth either carcinoma or fibrosarcoma may develop. I have observed several cases in which the histologic features of adenofibroma were well preserved, and in one portion the epithelium appeared in actively proliferating strands invading the stroma. Fibrosarcoma arising in adenofibroma is a definite type of sarcoma of the breast, recognizable by gross and histologic features, and apparently running a clinical course different from other sarcomas of the breast. Fifteen cases of this have been collected from the material of the laboratory of the Huntington Hospital and from that of the Palmer Memorial Hospital, accumulating during the last ten years at the former hospital, and during the last two years at the latter.

The fifteen cases are tabulated in the appended chart. It will be seen that seven cases, 1, 4, 6, 11, 13, 14 and 15, were found to present features that enabled me to make the histologic diagnosis of adenofibrosarcoma. This proportion cannot be considered significant because the number of cases is small, but it is noteworthy that Ewing [1] stated that true adenosarcoma of the breast is rare. On the other hand, Deaver and MacFarland [6] in their compilation of cases of sarcoma of the breast gave a total of 193 cases of adenosarcoma occurring in a group of 838 sarcomas of the breast. In the series reported by Gross,[7] 33 per cent of a total of 156 cases of sarcoma of the breast are termed "adenoid," and may be presumed to belong to the category of adenosarcoma. Finsterer [8] reported forty cases, eighteen of which he considered as "cystosarcoma." This group, too, may correspond to the special variety of sarcoma under discussion.

The relative frequency of this type of tumor is of about the same magnitude in my series as in the aforementioned series reported by others.

It is, perhaps, noteworthy that the only two cases of sarcoma of the breast in the records of the Palmer Memorial Hospital are both cases

4. Cheatle, S. L.: Mastitis, Cysto-Adenoma and Adenoma of Breast, Arch. Surg. **17**:535 (Oct.) 1928.

5. Williams, R.: M. Chron. **19**:289, 1894.

6. Deaver and MacFarland: The Breast, Philadelphia, P. Blakiston's Son & Company, 1917, p. 372.

7. Gross, S. W.: Sarcoma of Female Breast, Am. J. M. Sc. **94**:17, 1887.

8. Finsterer, J.: Ueber das Sarkom der weiblichen Brustdrüse, Deutsche Ztschr. f. Chir. **86**:352, 1907.

of adenofibrosarcoma. I consider this to be in accordance with the development of these tumors in that a high proportion of neoplasms seen at this hospital are in the early stages. These cases would, in my view, represent examples of early sarcoma of the breast at a stage in which the remains of the original mixed structure can be recognized.

Of the group of fifteen patients, the youngest was 29 years old and the oldest 75. The ages of the seven with adenofibrosarcoma ranged from 29 to 65. The age incidence does not seem to have special significance, apart from the fact that a benign adenofibroma of long standing may become sarcomatous, and this interval brings the patient into the region of middle age. In the total group, the duration of disease as given by the patient's history varied from three weeks to twenty years. In the included special group, the variation was also wide, so that no deductions from this factor seem possible.

All the tumors were of considerable size, even those the duration of which was reported as only three weeks. The smallest was said to be as large as a walnut, and the largest the size of a football. In the seven cases of adenofibrosarcoma, the tumors were of more nearly uniform size, ranging from the size of a goose egg to that of a football, with the intermediate ones fairly similar in magnitude. The fact that these tumors were all of considerable size seems of importance in two respects, first, in indicating a fairly long duration, probably more nearly uniformly long than was shown in the histories, and second, in furnishing a feature distinguishing adenofibrosarcoma from adenofibroma, which is usually not more than 5 or 6 cm. in diameter, and is most commonly reported clinically to be the size of a walnut.

Histologically, these cases have been divided into two groups, one with, and the other without, epithelial elements. All show more or less active fibroblastic tissue, with closely packed spindle-shaped cells, showing at least a few mitoses. In the group with epithelial elements, the arrangement is that seen in adenofibroma. There are clefts and ducts lined with cuboidal, columnar or stratified epithelium. The lumen is often more or less compressed and frequently contains desquamated epithelial cells. The fibroblasts are usually most closely packed immediately about the ducts, and in these regions most of the mitoses are to be noted. Farthest from the ducts, the fibrous tissue may be fairly acellular and the collagen dense and even hyaline. This patchy appearance, with active proliferation about the ducts and relative quiescence between them, is a fairly constant feature of these tumors early in their development. In one case, the intercellular substance was distinctly myxomatous (case 14). In another (case 6) there were only a few epithelium-lined spaces and between them was a uniformly cellular fibroblastic tissue, in which mitoses were numerous. This seemed histo-

logically a form intermediate between pure fibrosarcoma and adeno-fibrosarcoma, and clinically behaved more like the former than the latter.

Fig. 1.—Gross specimen in case 14, natural size. Only on the upper aspect is the encapsulation well defined.

A comparison of the histology and the clincal course tends to emphasize a difference between the two groups. In four of eight cases histologically fibrosarcoma, the subsequent course is unknown. In

three of the remaining four cases, the patients died of recurrence or metastases within eight months of operation. In one case of fibrosarcoma histologically of low malignancy, the patient remained well and was apparently free from recurrence six years after operation.

In the group diagnosed as adenofibrosarcoma there were seven cases. In five of these the patients were free of disease when last heard from, that is, from six months to seven years after the removal of the tumor. One (in case 1) had a recurrence four and one-half years after operation. This recurrent tumor was histologically considered to be fibrosarcoma, with no remaining trace of the original mixed structure. This observation, that a recurrence of an adenosarcoma may be a pure spindle cell sarcoma, was made by Finsterer.[8] The last case (no. 6) was intermediate in type histologically, and two recurrences appeared at intervals of six months and two months. Death occurred a year after operation, At this time, an abdominal tumor was said to be present, suggesting the possibility of metatasis.

The comparison in the clincal courses of cases of fibrosarcoma and adenofibrosarcoma may be made from the following cases.

M. C. (case 10), a housewife, aged 43, having seven children, came to the Huntington Hospital, April 1, 1924, because of swelling of the left breast. This had first been noted ten months previously as a lump the size of a hickory-nut, and growth had been constant. On examination, the left breast was found three times the size of the right. The skin was tense and marbled and seemed about to ulcerate. Axillary nodes were clinically negative. The tumor was movable, lobulated and elastic.

April 7, 1924: An amputation of the breast was performed by Dr. Robert Greenough. The axilla was not disturbed. In gross, the mass measured 7 inches (17.78 cm.) in diameter, and showed necrotic and cystic foci.

April 21, 1924: A small recurrence was noted in the granulating wound. Nine seeds each containing 1 millicurie of radium emanation were applied. May 8, 1924, the patient was discharged with two definite recurrences in the operative wound.

Microscopic study showed a pure fibrosarcoma, with large atypical spindle cells showing numerous mitoses, and a varying amount of intercellular substance. The patient died, Aug. 11, 1924, clinically with metastases, four. months after operation. Permission for postmortem examination was not obtained.

M. R. P. (case 13), a widow, aged 48, with one child, noticed a lump in the left breast, associated with stabbing pain, three weeks before coming to the Palmer Memorial Hospital. On examination, Sept. 27, 1927, the left breast was found twice the size of the right. In it was a hard, movable mass. The axillary glands were clinically negative.

Sept. 29, 1927: Radical mastectomy was done by Dr. L. S. McKittrick. In gross there was seen a well outlined tumor as big as a man's fist. This was histologically found to be made up of bundles of cellular fibroblastic tissue, with numerous mitoses scattered in the cells. Between the bundles were clefts and tubules lined by columnar and squamous epithelium. Healing was uneventful, and examination at subsequent visits showed a smooth scar, without evidence of recurrence.

It is to be noted that in neither of these cases was there any involvement of the axillary lymph nodes, nor was it reported as having been observed in any of the other cases of sarcoma of the breast. It would seem that there is no reason, therefore, for using the more difficult operation in cases of sarcoma. Finsterer [8] also found no metastases

Summary of Cases of Sarcoma of Breast

Case	Age	Duration	Size	Histologic Character	Clinical Course
1	61	2 years	"Football"	First specimen, adenofibrosarcoma; second, pure fibrosarcoma	Recurrence 4 years after removal of first tumor
2	..	.....	.....	Infiltrating bundles of spindle cells, with numerous mitoses; fibrosarcoma	Unknown
3	47	2 years	.....	Infiltrating bundles of spindle cells, with numerous mitoses; fibrosarcoma	Unknown
4	65	.....	.....	Cellular fibroblastic tissue in which were spaces lined by epithelium; adenofibrosarcoma	Well and healed
5	60	5 months	"Egg"	Spindle cells and giant cells, with many mitoses; fibrosarcoma	Unknown
6	48	5 months	"Goose Egg"	A few epithelium-lined spaces, in very cellular fibroblastic tissue with mitoses	Recurrence twice and death 1 year later
7	40	9 years	5 in., diameter	Spindle cells, with abundant collagen and few mitoses; fibrosarcoma	Well and healed
8	53	5 months	10 in., diameter	Spindle cells, with many mitoses and little collagen; fibrosarcoma	Metastases; died 8 months later
9	50	6 weeks	"Walnut"	Infiltrating cellular fibroblastic bundles, many mitoses; fibrosarcoma	Died 6 months after operation
10	43	1 year	17 cm., diameter	Closely packed spindle cells, with mitoses; fibrosarcoma	Recurrence and death in 4 months
11	29	5 months	.....	Numerous epithelium-lined clefts; cellular fibrous tissue with many mitoses	Well and healed
12	75	3 weeks	.....	Spindle cells and giant cells with mitoses	Unknown
13	48	3 weeks	"Fist"	Epithelium-lined clefts with cellular fibrous tissue showing mitoses; adenofibrosarcoma	Well and healed
14	51	6 months	"Orange"	Same as in case 13, with myxomatous fibrous tissue; adenofibrosarcoma	Well and healed
15	51	20 years	"Child's head"	Epithelium-lined clefts with fibrous tissue showing foci of condensation, with mitoses; adenofibrosarcoma	Well and healed

to lymph nodes in his series of forty cases. His statistics are also of interest as regards prognosis in the presence of various types of sarcoma. He classified eighteen of forty cases as "cystosarcoma," a term which appears suggestive of the group I have separated as cases of adenofibrosarcoma. He found twelve of the patients in these eighteen cases free of disease, one with a recurrence, five dead of intercurrent

disease and none with metastases, when they were reexamined over periods of several years after operation. These cases contrast with the ones he classified as cases of round and spindle cell sarcoma; in

Fig. 2.—Actively proliferating focus in section from case 13; ×300. Several mitotic figures may be distinguished in the paler portion of the field.

which only five of the twenty-one patients were free from disease, nine showed recurrences, three showed metastases and the condition of the rest were unknown.

From this series of cases, it appears that there is a definite difference in malignancy between fibrosarcoma and adenofibrosarcoma, apart from the ordinary criteria of cell activity in the fibroblasts themselves, and that the latter is considerably less malignant than the former. Two explanations appear possible. First, it may be that all sarcoma of the breast begins as adenofibroma and passes through an intermediary stage of adenofibrosarcoma. In this case, fibrosarcoma would be a more malignant tumor because of its longer standing and more advanced condition. The second possibility is that there exists a growth-restraining influence, perhaps, mutual, between the epithelial and connective tissues, and that this is sufficient to make the mixed tumor a less malignant one. The fact that an intermedite form of tumor occurred in this series is in favor of the former explanation. It has long been observed clinically that small tumors of the breast, such as are found on removal to be adenofibroma, if left undisturbed, may after months or years begin to grow and reach enormous size. Such tumors form the large ulcerated, fungating sarcomas of the breast occasionally seen. The fact that recurrences of adenosarcoma are known to appear as fibrosarcoma is further evidence that this graduation from benign to more and more malignant forms takes place.

I should consider the presence of epithelial elements in a sarcoma of the breast as significant in indicating an early stage of malignancy. It seems probable that if such tumors were allowed to grow uninterruptedly, the fibroblastic element would finally completely displace the epithelium. Consequently, it seems that the preservation of the basement membrane of the epithelium is just as much an indication in sarcoma that the fibroblasts have not yet grown beyond bounds, as it is in chronic mastitis that the epithelial hyperplasia has not become carcinoma.

CONCLUSIONS

In the heterogeneous group of sarcomas of the breast, the adenofibrosarcoma is a distict type. Adenofibrosarcoma is a comparatively frequent variety of sarcoma of the breast. It is probable that adenofibrosarcoma arises from a preexisting adenofibroma, and that it may progress to or appear in recurrences as pure fibrosarcoma. Thè prognosis in cases of adenofibrosarcoma is fairly good, and seems considerably better than in cases of pure fibrosarcoma.

THE THYMUS GLAND IN LYMPHATIC LEUKEMIA

ITS BEARING ON THE HISTOGENESIS OF THE SMALL THYMIC CELLS *

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Since Kölliker,[1] in 1879, drew attention to the epithelial anlage of the thymus gland in the branchial clefts, there has been considerable study in an attempt to determine the definite histogenetic nature of the organ. Yet many facts regarding the histogenesis of the thymus gland are still the subject of dispute, and this despite many excellent contributions that have been made on this subject.

In the course of an investigation of the thymus gland under certain pathologic conditions, I became particularly interested in the histogenesis of that component of the thymic parenchyma variously described as the "small thymic cells" or the "thymic lymphocytes." The histogenetic relationship of these cells to the original epithelial anlage has been the crux of a controversy which has attracted considerable comment, but which is, nevertheless, still settled.

HYPOTHETICAL SOURCES OF SMALL THYMIC CELLS

It may be said that two theories are at present most commonly held with reference to the source of the small thymic cells. According to one of the two theories, these cells are considered of mesodermal origin, whereas, according to the other, they are derivatives of the original epithelial anlage of the branchial entoderm. The studies of Maximow,[2] Pinner,[3] Danchakoff,[4] and Pappenheimer [5] all have tended

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* Work done in the Section on Pathologic Anatomy, the Mayo Clinic.

1. Kölliker, Albert: Entwicklungsgeschichte des Menschen und der höheren Thiere, Leipzig, Wilhelm Engelmann, 1879, pp. 875-882.

2. Maximow, Alexander: Untersuchungen über Blut und Bindegewebe: II. Ueber die Histogenese der Thymus bei Säugetieren, Arch. f. mikr. Anat. **74**:525, 1909; IV. Ueber die Histogenese der Thymus bei Amphibien, ibid., **79**:560, 1912; V. Ueber die embryonale Entwicklung der Thymus bei Selachiern, ibid. **80**:39, 1912.

3. Pinner, Max: Zur Frage der kleinen Thymusrindenzellen, Frankfurt. Ztschr. f. Path. **23**:479, 1920.

4. Danchakoff, Vera: The Differentiation of Cells as a Criterion for Cell Identification, Considered in Relation to the Small Cortical Cells of the Thymus, J. Exper. Med. **24**:87, 1916.

5. Pappenheimer, A. M.: Further Studies of the Histology of the Thymus, Am. J. Anat. **14**:299, 1913.

to indicate the morphologic identity of the small thymic cells with the blood lymphocytes. However, this does not imply histogenetic unity of the thymic cells and the true blood lymphocytes. Indeed, as has already been pointed out by Danchakoff, studies by the method of tissue culture have indicated that the form of the cell can be influenced considerably by various environmental factors. Hence, cellular morphology and even cellular differentiation, which is only a further expression of a potentiality for morphologic variation, cannot be adduced as final proof of biologic or histogenetic identity among cells. Somewhat more direct studies to determine the origin of the small thymic cells have been made by Rudberg [6] and Johnson,[7] who have studied the regenerative process in the thymus gland following experimental involution. These investigators concluded that the reappearance of the "lymphocytes" following involution was dependent on the immigration of lymphocytes into the epithelial network. However, these conclusions seemed untenable to Gottesman and Jaffe,[8] who studied the regeneration of autoplastic thymic transplants in albino rats. They were unable to demonstrate any evidence of lymphocytic infiltration from without and felt that the small thymic cells arose in situ from the proliferation of the reticular epithelium. Many more detailed histologic studies of the thymus gland in young embryos of the human being and of lower animals have indicated the epithelial origin of these thymic cells. The extensive studies of Stöhr,[9] Prenant,[10] Nusbaum and Prymak,[11] and those of Bell,[12] all have shown rather strikingly that following the transformation of the solid epithelial organ into the reticular network, there appeared the small thymic cell by a process of budding from the reticular epithelial cells. The gradual transition between the epithelial cells of the reticulum and the small thymic cells was demonstrated, and the definitive thymic cell then was found to break loose from the syncytium, passing into the spaces of the latter and assuming its position as a part of the thymic parenchyma.

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6. Rudberg, H.: Studien über die Thymusinvolution: I. Die Involution nach Röntgenbestrahlung, Arch. f. Anat. Entwcklngsmechn. d. Organ., 1907, supp., pp. 123-124.

7. Johnson, Arvid: Studien über die Thymusinvolution. Die akzidentelle Involution bei Hunger, Arch. f. mikr. Anat. **73**:390, 1909.

8. Gottesman, Jessie M., and Jaffe, H. L.: Studies on the Histogenesis of Autoplastic Thymus Transplantations, J. Exper. Med. **43**:403, 1926.

9. Stöhr, P.: Ueber die Natur der Thymus-Elemente, Anat. Hefte **31**:407, 1906.

10. Prenant, quoted by Bell.

11. Nusbaum, Josef, and Prymak, Theodor: Zur Entwicklungsgeschichte der lymphoiden Elemente der Thymus bei den Knochenfischen, Anat. Anz. **19**:6, 1901.

12. Bell, E. T.: The Development of the Thymus, Am. J. Anat. **5**:29, 1906.

This brief review indicates the salient facts on which are based the two diametrically opposed theories now held most commonly with reference to the histogenesis of the small thymic cells. A detailed review of this subject is given by Pappenheimer [13] and by Bell.

OBSERVATIONS OF THYMUS GLAND IN LEUKEMIA

An indirect manner of approaching the study of this problem is to investigate the reactions of the thymus gland, and particularly of the small thymic cells, under pathologic conditions in which the predominant influence is exerted on the true lymphocytic elements in the lymph nodes, blood and so forth. Thus, a study of the reactions of the thymus gland in lymphatic and aleukemic leukemia seemed to us of importance in its possibility of throwing some light on the relationship of the thymic cells to the lymphocytic elements elsewhere in the body. On a priori grounds, it may be assumed that if the small thymic cells are true lymphocytes, having not only a morphologic resemblance to blood lymphocytes, but also a common histogenetic relationship to them, there would occur in lymphatic leukemia hyperplasia of the thymus gland step by step with hyperplasia of the lymphatic structures elsewhere in the body.

A review of the literature does not reveal any extensive study from this standpoint. The recorded cases in which thymic hyperplasia was observed in patients with leukemia have yielded contradictory results, apparently owing to the limited material which was available for study. The conclusions which have been drawn from these studies parallel the contradictory evidence on which they were based.

Thorpe [14] reported "an unusual case of thymic hypertrophy" in a boy, aged 7, who was suffering from lymphatic leukemia. The thymic enlargement, in this instance, was detected by means of a roentgenogram, and postmortem confirmation was not available. General examination revealed also marked enlargement of the liver and spleen, and there was significant enlargement of the lymph nodes. This author quoted De Sautelle and Wood,[15] who reported similar enlargement of the thymus gland in a case of lymphatic leukemia.

Poensgen [16] found a thymus gland weighing 60 Gm. in a boy, aged 16, with lymphatic leukemia; in another case, that of a man, aged 60, the thymus gland weighed 2 Gm. and histologically presented lymphocytic infiltration. From this he concluded that the thymus gland, participating in the lymphocytic reaction, must be a lymphoid organ.

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13. Pappenheimer, A. M.: A Contribution to the Normal and Pathological Histology of the Thymus Gland, J. M. Research **22**:1, 1910.

14. Thorpe, E. S.: An Unusual Case of Thymic Hypertrophy, Arch. Pediat. **44**:126, 1927.

15. De Sautelle, W. T., and Wood, R. B., quoted by Thorpe (footnote 14).

16. Poensgen, Fritz: Beitrag zur Frage der Wechselbeziehungen zwischen Thymus, Schildruese und lymphatischen System, Med. Klin., **2**:1504, 1913.

Hart [17] was unable to report on any cases of lymphatic leukemia in children, but he had observed marked enlargement of the thymus gland in pseudoleukemia and felt that this was due to the participation of the lymphoid components of the thymus gland in the generalized lymphoid hyperplasia.

Ordway and Gorham,[18] in a consideration of lymphatic leukemia, stated: "The thymus gland is usually large and may even show tumor-like nodules and infiltration of the surrounding tissue. Histologically, the change is comparable to that found in the lymph glands."

Kaufmann [19] stated: "In leukemia, marked enlargement of the thymus gland is occasionally seen, especially in acute lymphatic leukemia."

Ewing,[20] on the other hand, remarked: "It is a notable fact that in most cases of leukemia and pseudoleukemia the thymus gland fails to participate in the process." Schridde expressed essentially the same opinion. Schridde [21] expressed the belief that it has not been established in the literature that true thymic hyperplasia occurs in lymphatic leukemia. He was not able to observe more infiltration in the thymus gland than he observed in the skin, for example.

In view of the contradictory views expressed with regard to the occurrence of thymic hyperplasia in lymphatic leukemia, I undertook a study of this problem in a series of cases of lymphatic and aleukemic leukemia which had come to necropsy at the Mayo Clinic.

Between 1910 and 1929 there were thirty-two cases of acute and chronic lymphatic leukemia and aleukemic leukemia in which complete postmortem examinations were performed. In each case, the clinical and anatomic data established the diagnosis. The age of the patients varied from 2 to 73 years; ten of the cases were in children. The duration of the symptoms also varied within wide limits. On thorough analysis, it seemed desirable to classify the cases into two arbitrary groups, one in which there was no enlargement of the thymus gland and another in which there was apparent enlargement of the gland.

Leukemia Without Enlargement of the Thymus Gland.—Group 1 (table 1) comprised a series of twenty-eight patients, twelve of whom were aged less than 30 years. Seven of these patients were children. The ages in the entire group varied from 2 years to 73 years and the duration of symptoms ranged from five days to three years. All but seven of these patients presented, in addition to the other anatomic evidence of lymphatic leukemia, a blood picture characteristic of the

17. Hart, Carl: Thymusstudien: III. Die Pathologie der Thymus, Virchows Arch. f. path. Anat. **214**:1, 1913.

18. Ordway, Thomas, and Gorham, L. W.: Leukemia, in Oxford Medicine, New York, Oxford University Press, 1920, vol. 2, pp. 716-718.

19. Kaufmann, Edward: Pathology for Students and Practitioners, Philadelphia, P. Blakiston's Son & Company, 1929, vol. 1, p. 501.

20. Ewing, James; Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1928, pp. 967-977.

21. Schridde, H.: Thymus, in Aschoff, Ludwig: Pathologische Anatomie, Jena, Johann Ambrosius Barth, 1921, pp. 176-189.

disease. In the other cases there was not an absolute increase in the number of circulating leukocytes, but a marked preponderance of lymphocytes, many of which were of the embryonal, immature type seen in this disease. The latter type was designated aleukemic leukemia,

TABLE 1.—*Cases in Which the Thymus Gland Was Not Enlarged (Group 1)*

Case	Diagnosis	Age, Years	Sex	Duration of Symptoms, Mo.	Leukocytes	Lymphocytes, per Cent	Pathologic Changes Noted in Thymus Gland
1	Aleukemic leukemia.....	2	M	2	5,700	97	Marked degree of involution; weight 12 Gm.
2	Aleukemic leukemia.....	4	M	5	9,500	98	Marked degree of involution with fibrosis; weight 12 Gm.
3	Acute lymphatic leukemia	4	M	2.25	42,800* 54,800*	99	None
4	Acute lymphatic leukemia	6	F	5 days	.....	90	Involution and infiltration of interstitial tissue with lymphocytes; weight 3 Gm.
5	Aleukemic leukemia.....	8	M	1	3,400	97	None.
6	Aleukemic leukemia.....	8	M	2	3,500	71	Involution and infiltration of interstitial tissue with lymphocytes; weight 12 Gm.
7	Aleukemic leukemia.....	9	M	2	5,290	89	Involution and infiltration of interstitial tissue with lymphocytes; weight 10 Gm.
8	Acute lymphatic leukemia	19	M	4	3,800	..	None
9	Acute lymphatic leukemia	24	M	1.5	64,300 97,200	76	None
10	Acute lymphatic leukemia	27	M	6	23,200 364,000	99	None
11	Subacute lymphatic leukemia	28	M	9	63,400 161,000	90	None
12	Acute lymphatic leukemia	30	M	3.5 weeks	239,000 465,000	..	None
13	Acute lymphatic leukemia	39	F	1.5	29,800	76	None
14	Acute lymphatic leukemia	39	M	5	37,700	99	None
15	Aleukemic leukemia.....	40	M	3	10,000	85	None
16	Aleukemic leukemia.....	40	M	12	10,400	57	None
17	Acute lymphatic leukemia	42	M	1.75	140,000	79	None
18	Chronic lymphatic leukemia	45	M	24	300,000	93	None
19	Acute lymphatic leukemia	46	M	1.5	25,800	90	None
20	Chronic lymphatic leukemia	50	M	24	385,000	97	None
21	Chronic lymphatic leukemia	54	M	12	171,000 298,000	98	Marked degree of involution with infiltration of fatty areolar tissue by lymphocytes
22	Acute lymphatic leukemia	55	M	1	38,000	..	None
23	Chronic lymphatic leukemia	59	M	18	183,000	97	Complete involution
24	Chronic lymphatic leukemia	61	F	8	602,000	91	Practically complete involution with infiltration of fat by lymphocytes
25	Chronic lymphatic leukemia	63	M	36	313,000	94	Complete involution
26	Chronic lymphatic leukemia	63	M	15	174,000	96	None
27	Lymphatic leukemia.....	73	M	?	.....	98	None
28	Acute lymphatic leukemia	73	M	?	232,000	90	Complete involution

* Lowest and highest count.

since it presented all the characteristics of true leukemia, except for the absence of an increased number of circulating lymphocytes. In all instances there was found, grossly, hyperplasia of the lymphoid elements in the lymph nodes and spleen and in many cases lymphocytic collections scattered throughout the liver and kidneys.

The thymus gland was not found to be enlarged in any of these cases. In five cases, thymic tissue could not be seen grossly within

the thymic fat. Microscopic sections of the latter revealed only small collections of lymphocytes within a loose, fatty, areolar tissue. The epithelial reticulum had practically entirely disappeared, and corpuscles of Hassall could not be found. In five other cases, the thymus gland was markedly reduced in size; the weights ranged from 3 to 12 Gm. Histologically, these glands presented the appearance of advanced involution. The boundary between cortex and medulla had disappeared, and the entire gland appeared much less cellular than is usual. The cortex, particularly, showed a dearth of small thymic cells. So marked was this decrease in the number of the small thymic cells that in some glands the reticulum became prominent, giving the gland more of an

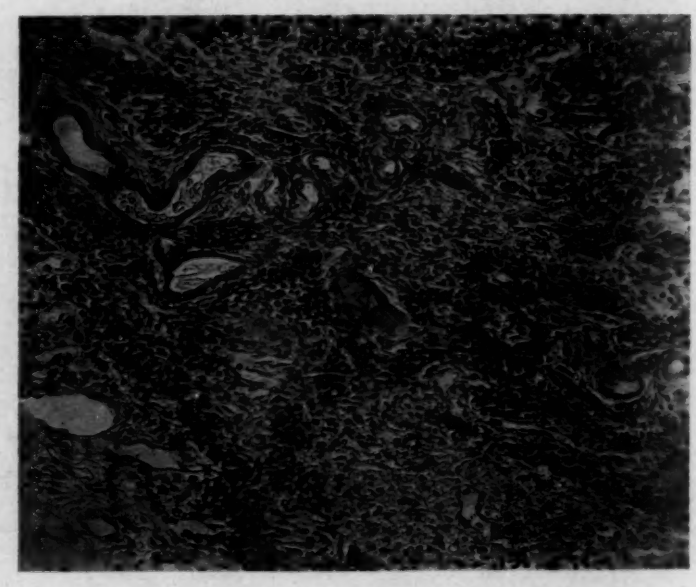

Fig. 1.—Section of thymus gland of a child, aged 4, who died from lymphatic leukemia. A marked degree of involution and fibrosis is present (× 175).

epithelial appearance. The interstitial tissue was markedly increased in amount and was composed of loose fibro-areolar tissue in which there were scattered varying numbers of small lymphocytes (figs. 1 and 2). In some sections, the fatty areolar tissue, which had entirely supplanted the original thymic parenchyma, showed numerous dense collections of lymphocytes in which remains of reticular cells or corpuscles of Hassall could not be seen. The fibrous capsule about the thymic fat body and sections of the posterior mediastinal wall revealed similar collections of lymphocytes (fig. 3), and this seems good evidence that the lymphocytes seen in some of these glands were merely a part of the general process of lymphocytic accumulation in tissues, which is char-

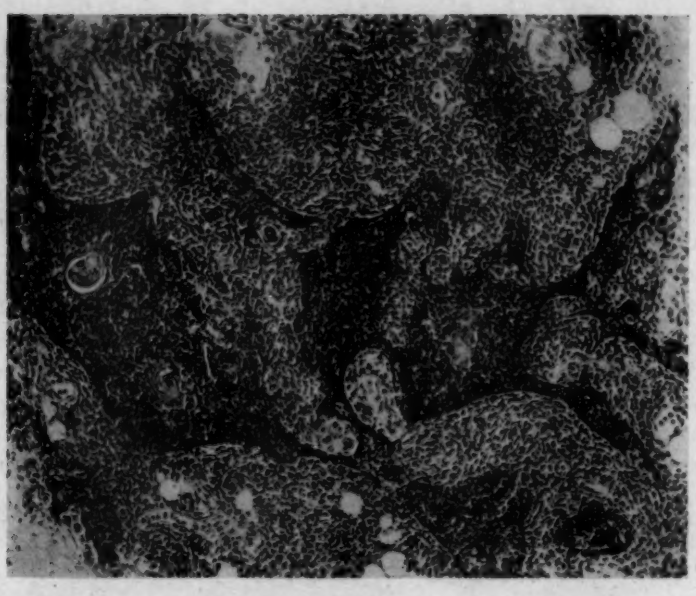

Fig. 2.—Section of thymus gland of a child, aged 6, who died of acute lymphatic leukemia. There is evidence of involution and infiltration of interstitial tissue with lymphocytes (× 150).

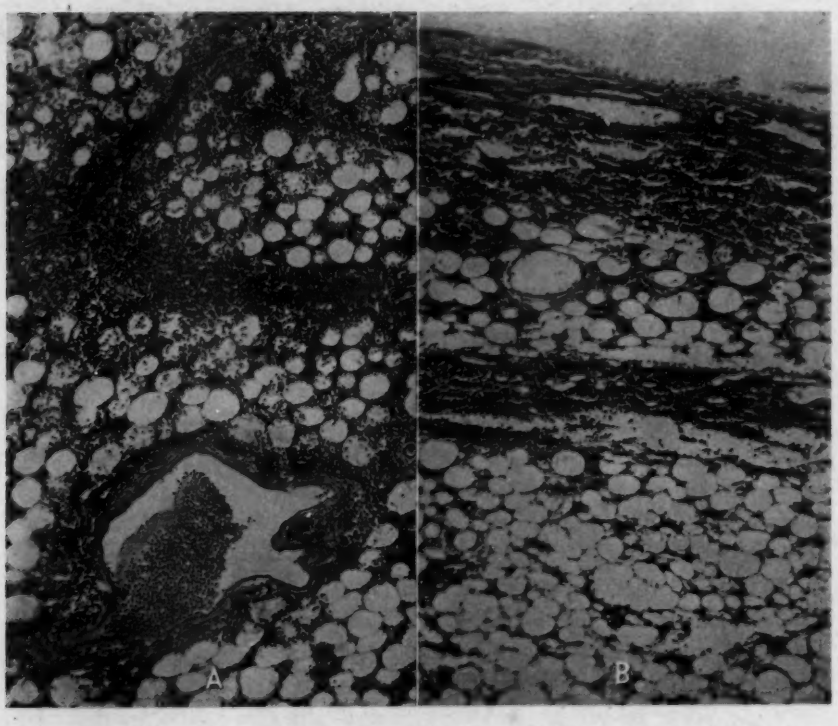

Fig. 3.—*A*, thymic fatty areolar tissue heavily infiltrated with lymphocytes in a man, aged 54, who died from lymphatic leukemia (× 100); *B*, section of posterior mediastinal wall of the same patient, showing a nearly proportionate degree of lymphocytic infiltration (× 100).

acteristic of the disease. In sections of glands in which involution was not complete, the corpuscles of Hassall appeared somewhat increased in number; this was probably a relative increase made apparent by the degeneration and disappearance of many small thymic cells. Most of the corpuscles showed hyaline degeneration, and only occasionally were newly formed corpuscles encountered. The entire process partook essentially of the nature of advanced involution such as one encounters in various disease processes and altered nutritional states. It is significant that the changes just described occurred as frequently in the glands of children as in those of adults, and in them the factor of involution from age had not yet come into play. In the remaining cases of this group, the thymus gland did not reveal gross abnormalities, and hence microscopic sections were available in only one case, that of a man, aged 50, who had had symptoms of acute lymphatic leukemia for

TABLE 2.—*Cases in Which the Thymus Gland Apparently Was Enlarged (Group 2)*

Case	Diagnosis	Age, Years	Sex	Duration of Symptoms, Mo.	Leukocytes	Lymphocytes, per Cent	Pathologic Changes in Thymus Mass
29	Aleukemic leukemia.....	5	M	5.5	2,800* 8,000*	70 92	Involution with fibrosis and diffuse lymphocytic infiltration; weight 50 Gm.
30	Acute lymphatic leukemia (leukosarcoma)	2	M	1	32,800	80 88	Involution with fibrosis and diffuse lymphocytic infiltration; weight 215 Gm.
31	Acute lymphatic leukemia (leukosarcoma)	23	M	4	297,000	94	Involution and lymphocytic infiltration; weight 470 Gm.
32	Acute lymphatic leukemia (leukosarcoma)	8	M	1	55,000 232,000	86 80	Involution with fibrosis and diffuse lymphocytic infiltration; weight 255 Gm.

* Lowest and highest counts.

from three to four months. The section of the thymus gland showed a marked degree of involution, with degeneration and disappearance of a large portion of the cortex, and a corresponding degree of replacement by fibrous tissue.

Leukemia with Enlargement of the Thymus Gland.—Group 2 (table 2) comprised a series of four cases, three in children and one in a man, aged 23. Three of these cases presented the picture of rapidly progressive acute lymphatic leukemia. In addition to the generalized tendency for lymphocytic accumulation within tissues, there was a tendency to maximal infiltration in only one or two sites, such as the mediastinal structures and kidneys. The result was huge enlargement of the kidneys and the mediastinal structures, the latter becoming matted together and appearing as if frozen to the lungs and pericardium (fig. 4). Because of this tendency to a sarcomatous type of proliferation in a condition which is essentially one of leukemia, the term leukosarcoma may well be applied as denoting perhaps the most malignant

form of true lymphatic leukemia. The anterior mediastinal mass in these cases was remarkable for its gross size. In the one case of aleukemia leukemia in this group the weight of the mediastinal mass was 50 Gm., while in the three cases of leukosarcoma the mediastinal tumors assumed huge proportions, weighing from 215 to 470 Gm. These appeared as huge mediastinal tumors which wrapped themselves about the pericardium and the great vessels. They were homogeneous, dull, white or yellowish white, and of rather firm consistence. The mass merged imperceptibly with the parietal pericardium to which it was adherent. When cut, it presented the same white appearance, and the

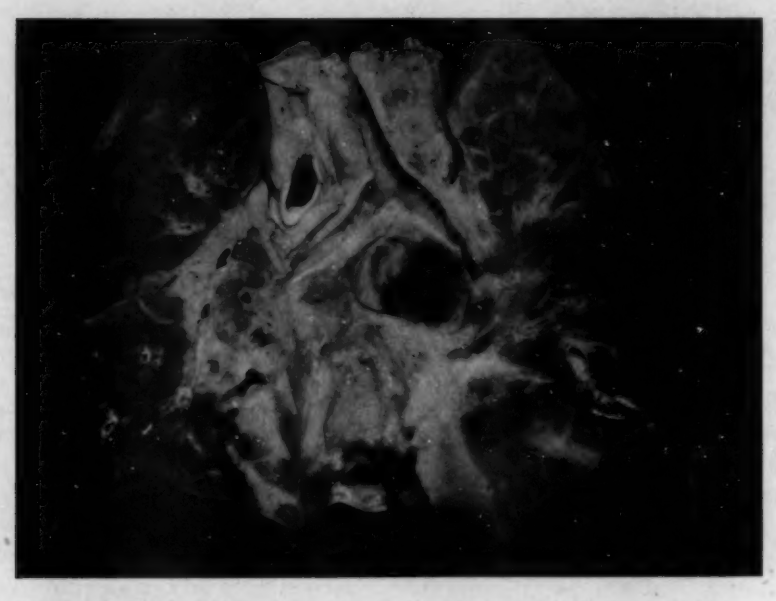

Fig. 4.—Sagittal section of anterior mediastinal mass, including the thymus, in a boy, aged 8, who died from leukosarcoma.

lines of cleavage between the thymus gland and its capsule and between the capsule and the parietal pericardium could not be distinguished.

Numerous sections taken from various parts of the mass could not be distinguished histologically. Material from the center, in the region of the thymus gland, showed essentially the same histologic structure as that taken from the capsule and the attached pericardium. The normal structure of the thymus gland was entirely obliterated; there was no reticulum and there were no corpuscles of Hassall. There were dense collections of lymphocytes throughout the entire mass and a rather abundant stroma composed of dense fibrous tissue. The lymphocytes varied considerably in size and some contained mitotic figures. Here and there the lymphocytes were densely aggregated in small groups which stood out in sharp contrast with the remainder of the parenchyma.

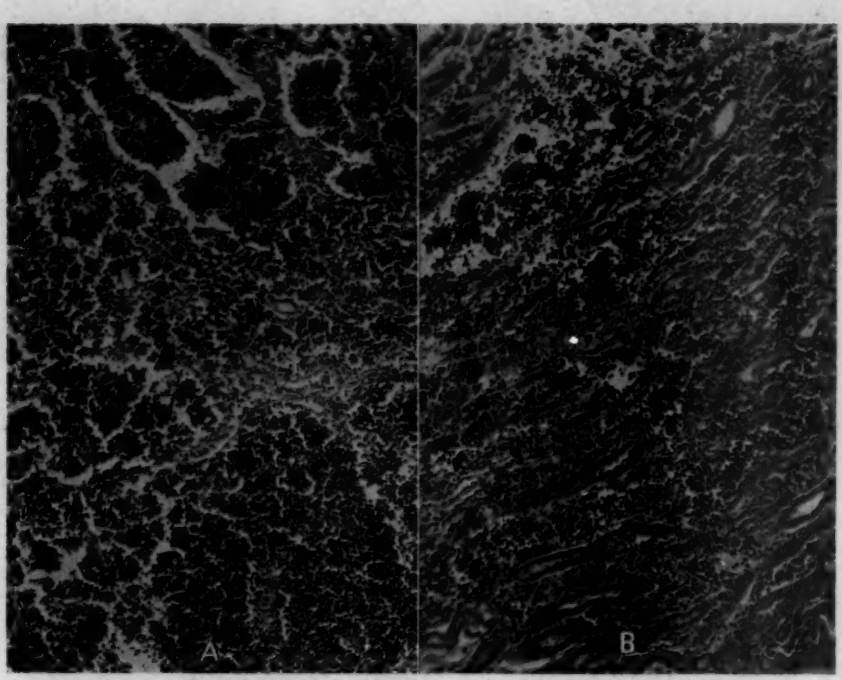

Fig. 5.—*A*, thymus of a child, aged 2, who died from leukosarcoma. Involution and fibrosis with diffuse lymphocytic infiltration are present (× 150); *B*, kidney of the same patient, also showing lymphocytic infiltration (× 150).

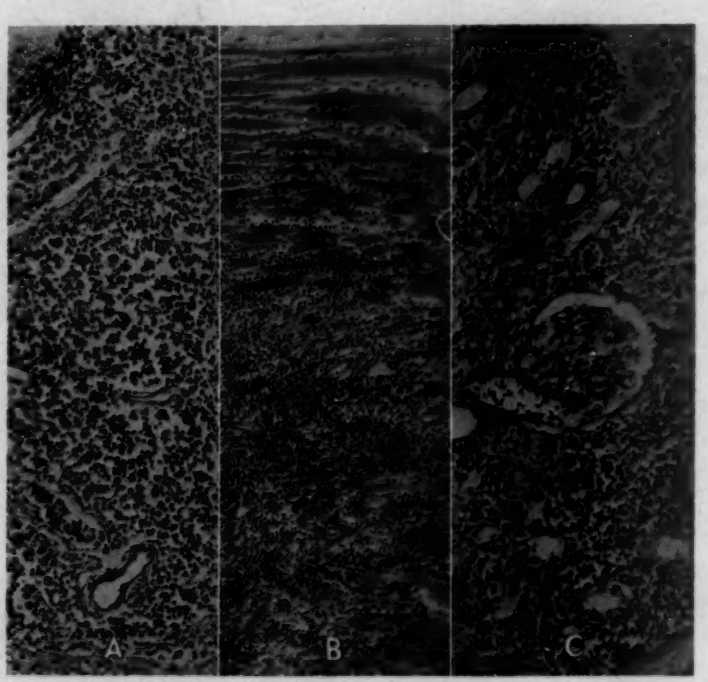

Fig. 6.—*A*, thymus gland of a child, aged 8. There is evidence of marked involution, with fibrosis and diffuse lymphocytic infiltration (× 175); *B*, diaphragm (× 150) and *C*, kidney of the same patient showing proportionate degrees of infiltration with lymphocytes (× 175).

In each of the three cases of leukosarcoma there was also tremendous enlargement of the kidneys, the liver and the spleen. The kidneys of one child, aged 2, weighed 545 Gm.; in another child, aged 8, both kidneys weighed 710 Gm., and in the third case, that of a man, aged 23, the kidneys weighed 1,046 Gm. The liver and the spleen were nearly proportionately enlarged. These organs were opaque and white, because of the dense collections of lymphocytes, and they presented evidence of extensive hemorrhage into their substance. Histologic sections of the kidneys and of the liver revealed extensive and dense infiltration with lymphocytes identical morphologically with those seen in the mediastinal mass. In part of the section, the infiltration with lymphocytes was so dense as entirely to obliterate the normal structures of the organ. Such fields presented a histologic appearance almost identical with that of the mediastinal mass, except that the latter contained a greater amount of interstitial tissue. The parenchymatous elements of the kidneys and the liver revealed extensive granular degeneration. Sections from other tissues, such as those of the gastrointestinal tract, and even the diaphragm, showed that they also were affected to a variable degree by the general infiltrating process (figs. 5 and 6). General lymphoid hyperplasia was present in all cases of the group.

COMMENT

In the entire series of thirty-two cases of various types of lymphatic leukemia, enlargement of the thymus gland was found in only four cases. In the remaining twenty-eight cases, the thymus gland either did not show notable abnormality or showed gross or histologic evidence of varying degrees of involution. That ten of the cases were in children emphasizes the fact that the involution was not always that due to physiologic retrogression associated with age, but that it was a reaction of the thymus gland to the disease process. It is not possible, of course, to say just what influences the thymus gland in leukemia. The altered nutritional state must be considered as one of the possible factors productive of the process of involution, since the relation of disturbed nutrition to pathologic involution of the thymus gland is now well established. What seems more interesting, however, is the striking involution of the thymus gland in a disease the essential feature of which is a tendency to hyperplasia of lymphoid tissues. This, indeed, brings into question the often quoted concept of the true lymphatic nature of the thymus gland and its small thymic cells. That the small thymic cells should react so unlike the lymphocytic elements elsewhere in the body suggests that they may be of inherently different nature, unless one postulates an overwhelming influence in leukemia productive of thymic involution and one sufficient to overcome the general stimulus

to lymphoid hyperplasia. I have no reason to believe that the latter is the case. The more direct interpretation of a different biologic nature of the small thymic cells and the blood lymphocytes seems more reasonable. I have already quoted evidence that has been adduced by many observers pointing to the epithelial source of the small thymic cells. The reactions of the thymus gland in leukemia also indicate a biologic difference; hence a different histogenetic source of the thymic cells from that of the blood lymphocytes does not seem unlikely.

The occurrence of an enlarged thymus gland in the four cases of leukemia would at first seem to militate against this hypothesis. However, the histologic features of these cases, too, point to an advanced degree of involution with secondary infiltration of the organ by lymphocytes. The lymphocytes in these glands appeared as densely aggregated in the connective tissue and capsule of the organ as in the parts normally occupied by the thymic parenchyma. Furthermore, the degree of infiltration in the thymus gland in the three cases of leukosarcoma paralleled that in other nonlymphatic organs, such as the kidney and the liver. It seems obvious from this that enlargement of the thymus gland in leukemia, when it does occur, represents merely a part of the tendency to infiltration of organs by lymphocytes that is so characteristic of this disease. In other words, the thymic enlargement in these cases does not represent true hyperplasia of the small thymic cells, but is due to nonspecific infiltration with lymphocytes, such as frequently affects the kidneys, liver, skin and other nonlymphatic structures in the body.

SUMMARY AND CONCLUSIONS

The thymus gland is rarely enlarged in lymphatic leukemia; the predominant tendency in this disease is in the direction of involution of the organ. When thymic enlargement does occur in leukemia, it usually parallels the degree of infiltration in other organs. Such enlargement of the thymus gland is not due to hyperplasia of the small thymic cells, but is due to infiltration by lymphocytes; the process is identical with that leading to infiltration of other nonlymphoid organs.

The reaction of the small thymic cells in lymphatic leukemia differs from that of the blood lymphocytes and suggests a difference in the biologic nature of the small thymic cells and the blood lymphocytes. A different histogenetic source of the small thymic cells from that of the blood lymphocytes may be inferred.

SERUM MENINGITIS *

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The stimulus for undertaking the investigation here recorded was the occurrence of several cases of aseptic meningitis discovered at autopsy in patients who had received serum injections into the spinal theca. Most of the cases were instances of fatal tetanus, but a case of cerebral hemorrhage clinically resembling meningitis in a young adult is also on record.

Most of the modern textbooks fail to mention the occurrence of aseptic meningitis following serum injections into the spinal theca; the few that do devote to it a few sentences without emphasis. In the literature references to the phenomenon are rather rare, but may be found even in articles of twenty years ago.[1] Recently interest in the subject has grown because induction of aseptic meningitis has been found of use in the treatment for schizophrenia and other mental states (Carroll, Kubitschek and Carmichael). A further experimental study in laboratory animals, with a review of as much of the literature as was available, seemed to be of timely interest.

Purves-Stewart [2] pointed out that "an abundant polynuclear pleocytosis" of the spinal fluid can be produced by the injection of sterile salt solution or sterile suspensions of colored particles into the spinal theca of monkeys. He mentioned no study of the lesions produced and relegated the fact to a footnote in the edition of his book published in 1911. Szecsi [3] made a study of the aseptic meningeal reaction to salt solution, which he reported in 1912.

Flexner and Amoss [4] noted the occurrence of aseptic meningitis in experimental animals following intrathecal administration of serum. They stated: "Introduction of sterile alien blood serum into the subarachnoid space causes an aseptic inflammation of mild degree that reaches its maximum in twenty-four hours and then subsides. The

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1. Widal et al., quoted by Frommel, E.: Schweiz. med. Wchnschr. **57**:873, 1927.

2. Purves-Stewart, Sir James: Diagnosis of Nervous Diseases, ed. 3, London, Arnold & Company, 1911.

3. Szecsi, S.: Ztschr. f. d. ges. Neurol. u. Psychiat. **60**:481, 1912.

4. Flexner, S., and Amoss, H. L.: J. Exper. Med. **20**:249, 1914; **25**:525, 1917.

inflammation is marked by emigration into the pia-arachnoid, ventricles and choroid plexus of polymorphonuclear leucocytes chiefly and by the escape of plasma." These investigators later found that, in addition to alien serum, normal homologous serum, physiologic solution of sodium chloride, Ringer's solution and sometimes even homologous spinal fluid caused a meningeal reaction when injected into the spinal theca. They were producing aseptic meningitis in monkeys to cause localization of poliomyelitic virus in the nervous system following intravenous inoculation. Flexner [5] stated that aseptic meningitis is of negligible importance in the treatment for meningitis.

The pharmacologic effect of intrathecal injections of serum is set forth in a masterful exposition by Auer.[6]

Ingleton [7] reported that there was a rather constant cellular reaction in the spinal fluid following intrathecal injection of antitetanic serum in cases of active tetanus. He withdrew second samples of fluid twenty-four hours after the injections of serum and found an average cell count of 400 per cubic millimeter, with 95 per cent polymorphonuclears. Postmortem examination in fatal cases of his series showed definite aseptic meningitis of varying intensity in each instance.

Leishman and Smallman [8] mentioned that one of them saw specimens of spinal cords removed after death in cases of tetanus, in which there was definite evidence that meningitis had followed the lumbar punctures. Their idea seemed to be that the meningitis was the effect of contamination carried into the theca by the needle. It apparently did not occur to them that the reaction could be aseptic and directly the result of the injections of serum.

The history of the "Medical Department of the U. S. Army in the World War" [9] reprints instructions given to medical officers concerning tetanus in which it is mentioned that intraspinal serum treatment may be followed by turbid spinal fluid, which is of relatively slight importance.

Ayer [10] in a study of the pathology of experimental meningitis discussed aseptic meningitis in some detail. He stated that aseptic menin-

5. Flexner, S.: Mode of Infection, Means of Prevention and Specific Treatment of Epidemic Meningitis, Monograph of the Rockefeller Institute, New York, 1917.

6. Auer, J.: J. Exper. Med. **21**:43, 1915.

7. Ingleton: J. Roy. Army M. Corps **26**:234, 1916.

8. Leishman, Sir W. B., and Smallman, A. B.: Lancet **1**:131, 1917.

9. The Medical Department of the U. S. Army in the World War, Washington, D. C., Government Printing Office, 1927, vol. 11, pt. 1, p. 284.

10. Ayer, J. B.: A Study of Experimental Meningitis: 2. Pathological Study of Experimental Meningitis from Subarachnoid Inoculation, Monograph of the Rockefeller Institute, no. 12, March 25, 1920.

gitis may be expected following intrathecal injection of almost any foreign substance, and mentioned among other substances capable of causing this reaction autogenous, homologous and heterologous serums. In his article, he gave two protocols showing the acute and residual effects of intrathecal injections of serum into animals. His results are in no way different from those recorded in this article. Ayer's experiments are the only ones found that represent a careful investigation of serum meningitis per se.

Weed and Wegeforth [11] studied the effects of irrigations of the subarachnoid space. Physiologic solution of sodium chloride was found to be much more toxic in irrigations than a modified Ringer's solution.

Wegeforth and Essick [12] found that various antiseptics caused an aseptic meningitis. This observation is in agreement with the results of other workers who have studied this phase of the subject.

Wertheimer [13] reported a case of aseptic meningitis following spinal anesthesia.

Stone [14] mentioned the occasional occurrence of "so-called" serous meningitis following intravenous injection of alien serum. He did not seem to relate the meningeal reactions to intrathecal injections of serum. Shaw and Shapland [15] spoke of "tetanism in meningitis" and cited two cases which, from the histories given, might well have been tetanus with meningitis secondary to intraspinal medication.

Boyd [16] cited the work of Purves-Stewart and of Ingleton in mentioning that aseptic inflammation of the meninges occurs.

Kimura [17] used Flexner's method of producing aseptic meningitis with serum in a study of the penetration of drugs and biologic products into the spinal fluid.

Young and Alpers [18] found a relatively small cellular increase in the cells of the cerebrospinal fluid following the injection of Swift-Ellis serum into ventricles, cistern and lumbar sac.

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11. Weed, L. H., and Wegeforth, P.: J. Pharmacol. & Exper. Therap. **13**: 317, 1919.

12. Wegeforth, P., and Essick, C. R.: J. Pharmacol. & Exper. Therap. **13**: 335, 1919.

13. Wertheimer, P.: Lyon chir. **19**:387, 1922.

14. Stone, W. J.: Treatment of Tetanus, J. A. M. A. **78**:1939 (June 24) 1922.

15. Shaw, H. B., and Shapland, C. D.: Brit. M. J. **1**:183, 1923.

16. Boyd, W.: The Cerebrospinal Fluid, New York, The Macmillan Company, 1920.

17. Kimura: Japan M. World **3**:67 (April 15) 1923.

18. Young, A. W., and Alpers, B. I.: The Protein and Cellular Content of Cerebrospinal Fluid: Changes in Fluid Following Intraspinal, Intracistern and Intraventricular Therapy with Swift-Ellis Serum, Arch. Neurol. & Psychiat. **12**: 537 (Nov.) 1924.

In his study of meningeal irritability, Kasahara [19] investigated the effect of various serums and "physiologic" solutions on the cell count of the cerebrospinal fluid in rabbits. He found that fresh serum called forth a greater cellular response than inactivated serum, and that heterologous spinal fluid was less irritating than any of the "physiologic" solutions. He did not record cultures of the spinal fluids and made no study of the lesions that he produced.

Golla and Herrmann [20] noted that aseptic meningitis follows injection of air into the subarachnoid space. Their studies showed no similar effect following lumbar puncture alone.

Frommel [21] discussed at length aseptic serum meningitis occurring in the course of treatment for cerebrospinal fever. He cited the work of several French investigators. According to him there are two kinds of reactions to serum on the part of the meninges, difficult to differentiate in practice. The first is the simple reaction of the theca to a foreign substance of any sort, and the second is a local manifestation of a general sensitive state (anaphylaxis) occurring only after a latent period following sensitization. He regarded serum meningitis as definitely harmful to the prospects of a patient receiving treatment for infectious meningitis, and advised cessation of intrathecal injections of serum when the spinal fluid has been cleared by thorough treatment at the beginning of the disease. Frommel deplored the general lack of recognition of this condition in the course of meningitis of infectious origin.

In the last few years, several reports of the therapeutic effect of aseptic meningitis in human beings suffering from mental disorders, particularly schizophrenic states, have appeared in American journals.

The first report of such work is in an article by Carroll [22] published in 1923. Several investigators have taken up this work.[23] The experiments of Kubitschek and Carmichael [24] are the most recent and represent the most thorough study of the physical aspects of this form of treatment. Inactivated horse serum was injected intraspinously into patients suffering from dementia praecox in doses of 20 cc. after a somewhat larger amount of fluid was drawn off. The cell count of the spinal fluid invariably rose, reaching its height in from twenty-four to forty-eight hours. After a single injection, from 200 to 5,500 cells per

19. Kasahara, M.: Experimental Study in Meningeal Irritability, Am. J. Dis. Child. **28**:407 (Oct.) 1924.

20. Golla, F., and Herrmann G.: Med. Klin. **22**:1951, 1926.

21. Frommel, E.: Schweiz. med. Wchnschr. **57**:873, 1927.

22. Carroll, R. S.: New York M. J. **118**:407 (Oct. 3) 1923.

23. Carroll, R. S.; Barr, E. S.; Barry, R. G., and Matzke, D.: Am. J. Psychiat. **4**:673, 1924-1925.

24. Kubitschek, P. E., and Carmichael, F. A.: Am. J. Psychiat. **8**:27, 1928.

cubic millimeter were found, mostly polymorphonuclears in the early stage of the reaction. After seven or more days, the count was much reduced, and most of the cells were lymphocytes. A leukocytosis of the blood ran parallel with that in the spinal fluid. Second and subsequent injections after a week or more were associated with more severe types of reaction in some cases in which the spinal fluid pressure and cell count were unusually high (40 mm. of mercury and 13,000 cells). The patients with such severe meningeal reactions were always relieved by cistern puncture. Systemic anaphylactic manifestations were controlled with epinephrine.

Recently, Bagley [25] reported experiments on the effect of blood in the cerebrospinal fluid. He found a definite microscopic cellular reaction to the presence of the blood, most marked at the site of the injection (subdural). His experiments, however, dealt with the more prolonged and severe types of bleeding into the subarachnoid space.

MATERIALS AND METHODS

Dogs were used in all of the experiments here recorded. They were considered most suitable because of the ease with which the cisterna magna can be reached with a needle. Lumbar puncture in the dog is unsatisfactory, because the spinal cord extends to the first sacral vertebra.

For the operation of cistern puncture, the animal was tied to the table with the belly down, and the back of the head and neck were shaved in a wide area. Ether anesthesia was used in every case. No postoperative pulmonary complications were encountered following the anesthesia, probably because in no case was the anesthesia carried on for more than ten minutes. The shaved area was cleansed with iodine and alcohol or with iodine and 5 per cent phenol. The punctures were done with a 19-gage needle attached to an all-glass syringe. The cisterna could in most cases be entered quickly and easily by thrusting the needle through the skin at the level of the transverse process of the atlas, pointing forward at an angle of 45 degrees to the perpendicular. The cisterna is usually reached at a depth of from 1.5 to 2 cm. depending on the size of the animal used. As a precautionary measure to prevent injury to the brain stem as a result of inserting the needle too far, slight suction is made with the syringe just as it enters the cistern.

From 5 to 8 cc. of clear cerebrospinal fluid can be removed from a normal animal without causing any unfavorable symptoms. In making injections into the cisterna magna, it is useful to employ a three-way stopcock to which the needle and two syringes can be connected, one empty syringe to aspirate the fluid and one syringe containing the fluid to be injected. When a suitable amount of fluid is withdrawn, the injection is made by turning the stopcock and allowing the injected liquid to run into the cisterna by its own weight. Aspiration of the cerebrospinal fluid is necessary, because with the animal ventral side down gravity does not help the fluid to flow upward through the needle.

Cell counts and globulin determinations were done on all but grossly bloody fluids. Chemical blood tests (Meyer's) were done on all fluids. When a trace

25. Bagley, C.: Blood in the Cerebrospinal Fluid: Resultant Functional and Organic Alterations in the Central Nervous System, Arch. Surg. **17**:18 (July) 1928.

of blood was found chemically, counts of both red and white cells in the spinal fluid were made. The most convenient way to do this is to use a double-ruled counting chamber and count the cells in laked (acetic acid) and unlaked specimens of spinal fluid, the difference representing the number of red corpuscles. Counts of less than 1,500 red blood cells per cubic millimeter were considered of no significance, since less than $\frac{1}{3,000}$ the volume of fluid withdrawn could be blood, and this probably was derived from tissues through which the needle must pass to reach the cistern. Under ordinary circumstances, with a ratio of about 1:500 of white cells to red cells in the blood, 1,500 red corpuscles per cubic millimeter account for only 3 leukocytes in a similar volume, a number which is of no significance in a spinal fluid cell count. In addition, all abnormal and most normal fluids were cultured in two or three different mediums to rule out septic meningitis.

Most of the experiments consisted of a single injection of serum or other given fluid followed in a variable period by a second puncture to discover the changes wrought by the injection. The animal was then killed, and the brain removed. Some experiments involved more than two punctures, and some animals were saved for three or more weeks after unsatisfactory experiments and then used again. None of the animals suffered any ill effects from either cistern puncture or injection. Occasionally, an animal was overcome by the anesthetic and had to be resuscitated with artificial respiration. Removal of the brain was facilitated and rendered bloodless by bleeding the animal from the femoral artery before death or by cutting the great vessels of the thorax and allowing the animal to bleed internally.

The quickest and simplest way to remove the dog's brain is to make a trephine hole in the temporal region of the skull and then rongeur the skull away from the brain. The organ is then lifted out while the nerve roots are cut from before backward. All brains were fixed in toto in formaldehyde (4 per cent) for a varying period, not less than two weeks. Paraffin sections were made of pieces cut from at least four different regions and stained by the usual hematoxylin-eosin method.

The serums used for injection were the commercial products of reliable manufacturers, identical with the products used therapeutically in human beings.

EXPERIMENTS AND RESULTS

Control Experiments.—As control experiments, simple cisternal puncture, reinjection of spinal fluid and injection of salt solution were done. Simple cisternal puncture and withdrawal of cerebrospinal fluid had only a slight effect on the spinal fluid drawn off subsequently, and this was very likely secondary to slight bleeding at the point of the first puncture after the needle was withdrawn.

Experiment 2.—January 10, at 3:30 p. m., with the dog under ether anesthesia, cistern puncture yielded 7 cc. of crystal clear fluid. No injection was made. A culture of the fluid was negative. The fluid contained five lymphoid cells per cubic millimeter. No symptoms resulted from the puncture. January 11, at 8 a. m., with the dog under ether anesthesia, a second puncture was done; the first portion of fluid obtained was slightly blood tinged, but after this clear fluid with a faint yellow color was obtained. A culture of the fluid was negative. It contained 34 cells per cubic millimeter, chiefly of lymphoid type. The animal was bled 500 cc. from the femoral artery, and sufficient ether was administered to kill it. The brain was removed and fixed in formaldehyde. Slight hemor-

rhage was caused in the cerebellopontile angle in removing the brain. No gross abnormalities could be detected. Microscopically, the brain and meninges had no pathologic characteristics.

Reinjection of the spinal fluid just withdrawn caused a slight transient reaction in the spinal fluid. This reaction was more than might be expected, but the experience of many punctures in these animals indicates that no intrathecal injection can be made without causing some reaction.

EXPERIMENT 16.—February 14, at 3 p. m., with the animal under ether anesthesia, cistern puncture yielded 5 cc. of crystal clear fluid, of which 2.5 cc. was reinjected slowly. The fluid contained 5 cells per cubic millimeter of lymphocytic type. A chemical test for blood was negative. February 15, at 3 p. m., with the animal under ether anesthesia, cistern puncture yielded 5 cc. of slightly turbid fluid. A culture of the fluid was negative. The fluid contained 750 cells per cubic millimeter, made up of equal numbers of polymorphonuclears and lymphocytes; 240 red corpuscles per cubic millimeter were present, and tests for globulin and blood were positive. February 18, with the dog under ether anesthesia, cistern puncture yielded 5 cc. of clear colorless fluid. It contained 8 lymphocytes per cubic millimeter. The chemical test for globulin was negative, and the test for blood faintly positive. The animal was saved for further experiment, since it was felt that sections would show no significant changes.

Injection of physiologic (0.9 per cent) solution of sodium chloride caused a more profound reaction than reinjection of spinal fluid.

EXPERIMENT 14.—February 4, at 3:30 p. m., with the dog under ether anesthesia, cistern puncture yielded 5 cc. of crystal clear fluid; 5 cc. of saline solution (0.9 per cent) was injected. The fluid contained 10 cells per cubic millimeter. Chemical tests for globulin and blood were both negative. February 5, at 8 a. m., with the animal under ether anesthesia, cistern puncture yielded 6 cc. of slightly turbid, colorless fluid. The thoracic vessels were cut to cause internal bleeding. The brain was removed and fixed in formaldehyde. The cistern fluid contained 766 leukocytes per cubic millimeter, made up of equal numbers of polymorphonuclears and lymphocytes, and 64 red corpuscles per cubic millimeter. A faintly positive chemical test for blood, and a positive test for globulin were noted. The animal had shown no symptoms in the course of the experiment. Microscopic sections of the brain and meninges showed a moderate invasion of the pia by polymorphonuclear leukocytes and lymphocytes and some exudation of these cells into the subarachnoid space over both cortex and base. There was some cellular invasion of the stroma of the choroid plexus of the fourth ventricle and moderate denudation of the epithelium covering the plexus in several places. Similar results were obtained in another animal subjected to a similar experiment.

Effect of Bloody Spinal Fluid.—Contrary to what might be expected, a puncture yielding grossly bloody fluid resulted in changes much less marked than those caused by salt solution.

EXPERIMENT 7.—January 22, in the afternoon, with the dog under ether anesthesia, cistern puncture yielded grossly bloody fluid. The animal reacted well from the anesthetic. In the morning, January 23, cistern puncture under ether anesthesia yielded 5 cc. of somewhat yellowish fluid containing many red corpuscles. A culture of the fluid was negative. The animal was killed by cutting of the thoracic vessels. The brain was removed and fixed in formaldehyde. Microscopic examination of various parts of the brain and spinal cord showed only slight infiltration of mononuclear cells and a few polymorphonuclears in the neighborhood of blood vessels. The choroid plexus appeared normal.

Horse Serum Injections.—Intrathecal injections of horse serum were found to produce a considerable leukocytosis in the spinal fluid within twenty-four hours. The reaction subsided in the next two or three days. A slight residual pleocytosis was found in one animal that had been spared, after three weeks. A second injection several weeks after a first injection of serum resulted in a milder type of reaction than did the first injection. (In connection with this fact, we are reminded of the dog's low susceptibility to anaphylactic shock.)

EXPERIMENT 13.—January 28, at 3 p. m., cistern puncture yielded 4 cc. of crystal clear fluid; 3 cc. of horse serum was injected. A culture of the fluid was negative. The fluid contained 15 lymphocytes per cubic millimeter. A chemical test for blood was faintly positive. January 29, at 8:15 a. m., with the dog under ether anesthesia, cistern puncture yielded 5 cc. of slightly cloudy fluid. More ether was administered, and the thoracic vessels were cut to kill the animal. The brain was removed and fixed in formaldehyde. The fluid contained 1,128 leukocytes per cubic millimeter, made up of equal numbers of polymorphonuclears and lymphocytes. No red corpuscles were present, and the chemical test for blood was negative. A test for excess globulin was positive. A culture of the fluid was sterile. Study of microscopic sections showed widespread infiltration of the pia and subarachnoid space with an exudate consisting mainly of polymorphonuclear cells, but including a few lymphocytes and mononuclears. The reaction was heaviest over the brain stem and cerebellum, as might be expected from a cisternal injection. The cerebellar cortex was found invaded by the inflammatory reaction, with slight destruction of brain substance in a few small areas. The choroid plexus showed moderate invasion of the stroma by lymphocytes.

EXPERIMENT 10.—January 8, at 3:30 p. m., with the dog under ether anesthesia, cistern puncture yielded 6 cc. of crystal clear fluid; 5 cc. of the horse serum was slowly injected. The animal reacted well from the anesthetic. A culture of the fluid was negative. The fluid contained 12 cells per cubic millimeter. A chemical test for globulin was negative. January 10, at 8:30 a. m., with the animal under ether anesthesia, cistern puncture yielded at first blood-tinged fluid and then crystal clear fluid. A culture of the fluid was negative. The chemical test for blood was negative; that for globulin, faintly positive. Thirty-nine cells per cubic millimeter were present, mostly lymphocytes. The animal was bled 750 cc. from the femoral artery, and enough ether given to kill it. The brain was removed and fixed in formaldehyde. Microscopic sections showed a mild meningeal reaction consisting of polymorphonuclear and mononuclear cells. The reacting cells were most numerous near blood vessels. There was increase in cellularity of the choroid stroma with distortion and denudation of portions of the choroid ependyma.

EXPERIMENT 6.—December 28, with the dog under ether anesthesia, cistern puncture yielded 5 cc. of slightly blood-tinged fluid, and 3 cc. of horse serum was injected. The animal reacted well from the anesthetic. A culture of the fluid was negative. January 31, at 8:30 a. m., with the dog under ether anesthesia, cistern puncture yielded clear fluid; 5 cc. of horse serum was injected. The fluid contained 22 lymphocytes per cubic millimeter. Tests for globulin and blood were negative. February 1, at 8:30 a. m., with the animal under ether anesthesia, cistern puncture yielded slightly turbid fluid. The thoracic vessels were cut, and sufficient ether given to kill the animal. The brain was removed and fixed in formaldehyde. A culture of the fluid was negative. The chemical

test for blood was negative; that for globulin, positive. There were 650 leukocytes per cubic millimeter, made up of equal numbers of polymorphonuclears and lymphocytes. Microscopic examination of the brain and meninges showed a moderate reaction consisting of polymorphonuclear and mononuclear cells in the pia. The reacting cells were frequently grouped in the neighborhood of the blood vessels. The most intense reaction was in the region of the brain stem. The stroma of the choroid plexus of the fourth ventricle showed increase in cellularity, but the ependyma was of practically normal appearance.

Therapeutic Serums.—Refined forms of horse serum, such as are used in the treatment for meningitis and tetanus, cause changes differing in no way from those caused by the unrefined horse serum.

EXPERIMENT 17.—February 26, at 3:30 p. m., with the dog under ether anesthesia, cistern puncture yielded 5 cc. of clear fluid; 3.5 cc. of tetanus antitoxin (2,100 units) was injected. The fluid removed contained 10 cells per cubic millimeter. Tests for blood and for globulin were negative. February 27, at 8:15 a. m., with the animal under ether anesthesia, cistern puncture yielded 4 cc. of slightly turbid, somewhat yellow fluid. The thoracic vessels were cut, and sufficient ether administered to kill the animal. The brain was removed and fixed in formaldehyde. A culture of the cistern fluid was negative. Leukocytes numbered 1,880 per cubic millimeter, polymorphonuclears constituting 60 per cent and lymphocytes, 40 per cent. Chemical tests for globulin and blood were both positive. It was believed that the blood was of no significance and had its origin outside the cistern. Microscopic sections showed moderately heavy invasion of the pia and subarachnoid space with leukocytes, over both cortex and brain stem. Some cellular exudate was found in the cerebral ventricles. There was increased cellularity of the choroid stroma.

EXPERIMENT 19.—February 19, at 3 p. m., with the dog under ether anesthesia, cistern puncture yielded 5.5 cc. of clear fluid; 5 cc. of antimeningococcus serum was injected. The fluid contained 8 cells per cubic millimeter. The chemical test for globulin was negative, and that for blood, faintly positive. February 20, at 8:30 a. m., with the animal under ether anesthesia, cistern puncture yielded slightly turbid fluid with a faint yellowish tinge. A culture of the fluid was negative. Leukocytes numbered 2,230 per cubic millimeter, and were made up of equal numbers of polymorphonuclears and lymphocytes; 2,500 red corpuscles per cubic millimeter were counted, and the test for blood was positive. The blood was considered an accidental contamination. February 21, at 2 p. m., with the animal under ether anesthesia, cistern puncture yielded almost clear fluid with a slight yellowish color; 172 leukocytes and 2,100 red corpuscles per cubic millimeter were counted. The chemical test for globulin was faintly positive.

Tricresol.—Tricresol was injected in one experiment because it is the most commonly used preservative for commercial serums. It produced a marked leukocytosis of the spinal fluid, but the histologic picture of the meninges showed a less marked reaction than that following injections of serum.

EXPERIMENT 15.—February 12, at 2 p. m., with the dog under ether anesthesia, cistern puncture yielded 7 cc. of clear fluid; 5 cc. of the spinal fluid was mixed with 1 cc. of 2.4 per cent cresol by means of the three-way stopcock (resulting mixture contained about 0.4 per cent cresol in spinal fluid) and the mixture injected. The spinal fluid contained 2 cells per cubic millimeter. February 13,

at 8:30 a. m., with the animal under ether anesthesia, cistern puncture yielded 6 cc. of slightly turbid fluid. The animal was killed, and the brain removed and fixed in formaldehyde. The fluid contained 1,365 leukocytes per cubic millimeter; 320 red corpuscles per cubic millimeter were counted, and the chemical test for blood was positive. The test for globulin was faintly positive. Microscopic sections showed a moderate reaction of polymorphonuclear cells and lymphocytes in the pia and some edema of the meninges over the cortex and brain stem. There was moderate invasion of the stroma of the choroid plexus of the fourth ventricle with polymorphonuclear leukocytes and lymphocytes, and some swelling and denudation of the ependymal covering of the plexus.

TABLE 1.—*Effect of Intraspinal Injection on the Cell Count of the Spinal Fluid*

Exper. No.	Substance Injected	Time After Injection	Spinal Fluid Cell Count, per Cu. Mm.	Histologic Reaction
8	Horse serum	17 hours	6,450	(No sections)
12	Horse serum	18 hours	978	Mild acute meningitis
13	Horse serum	17 hours	1,128	Acute meningitis
10	Horse serum	41 hours	39	Subsiding meningitis
4	Horse serum	5 days	.....	Mild subacute meningitis
6	Horse serum, 2d	24 hours	650	Subacute meningitis
5	Horse serum, 2d	24 hours	.....	Subacute meningitis
17	Antitetanus serum	17 hours	1,180	Acute meningitis
18	Antimeningitis serum	18 hours	5,220	Acute meningitis
19	Antimeningitis serum	18 hours	2,230	(No sections)
19	.....	40 hours	172	
20	Antimeningitis serum	5 days	34	Subacute meningitis
11	Simple puncture	17 hours	34	Normal, no reaction
16	Spinal fluid	24 hours	750	(No sections)
16	.....	4 days	8	
22	Spinal fluid	18 hours	250	Normal, no reaction
14	Salt solution	17 hours	765	Mild acute meningitis
9	Salt solution	17 hours	944	Mild acute meningitis
15	Cresol, 0.4 per cent	19 hours	1,365	Mild acute meningitis

TABLE 2.—*Experiment 21.—Effect of Multiple Intraspinal Injection of Antitetanus Serum on the Cell Count of the Spinal Fluid*

Date and Time	Antitetanus Serum Injected, Cc.	Spinal Fluid Cell Count, per Cu. Mm.
6/18, 8:15 a. m.	2.0	6
6/18, 2:45 p. m.	2.5	12,240
6/19, 8:30 a. m.	2.0	3,120
6/19, 3:00 p. m.	2.5	1,740
6/20, 8:00 a. m.	3.5	1,480
6/20, 2:30 p. m., killed	0.0	2,550
Microscopically, severe meningitis		

COMMENT

The results of these experiments are in agreement with those of other investigators. It was attempted to rule out all possibility of infectious meningitis by rigid adherence to aseptic technic and by culture of the cerebrospinal fluids obtained from the animals. It is hardly likely, therefore, that other than aseptic meningitis was present.

Various serums affected the meninges similarly. All called forth a polymorphonuclear reaction in the meninges and spinal fluid. The

intensity of the reaction varied from one animal to another and seemed, within limits, not to be related to the amount of serum injected or other controllable factors. It is significant that spinal fluid leukocytosis is always associated with a cellular reaction in the meninges. The experiments with serums rather definitely indicated that the cell content of the cerebrospinal fluid tends to diminish more rapidly and sooner than the meningeal reaction. That second injections had such small effect in dogs is not surprising, since the dog is not very susceptible to anaphylaxis.

The animals showed no unusual symptoms in cleancut experiments. Occasionally, it was noted that an animal was somewhat more active following injection of the serum, but usually the attendant was the only one who could note the difference in behavior. The serum caused no febrile reaction in the dogs.

Some animals, the protocols for which were not recorded, were subjected to multiple injections and punctures without adverse effect (table 2).

The effect of the injection of salt solution and cresol calls for no comment. The reaction to the reinjection of spinal fluid was so mild and transient that it is of little more than theoretical significance.

It is particularly in diseases such as tetanus or in diseases with meningeal symptoms but without actual meningitis that repeated intrathecal injection of large doses of serum may lead to confusion in diagnosis and at times to actual harm to patients so treated. Recognition of the effect of serum and other sterile fluids on the meninges will serve to dispel this confusion. In cases of infectious meningitis in which serum treatment is used, daily observation of the condition of the spinal fluid is necessary to regulate the therapy, for continued use of serum is not without harmful effects in the absence of infection necessitating its use. There is, however, no contraindication to the use of therapeutic serums intrathecally when the hazard of the disease is greater than that of the serum which promises relief.

CONCLUSIONS

Aseptic meningitis has received scant recognition in the textbooks and only occasional serious consideration in the literature.

Intrathecal injections of serum produce spinal fluid leukocytosis and an accompanying aseptic meningitis with great regularity in experimental animals.

This aseptic meningitis is of short duration and produces no permanent changes of any importance in the meninges or nervous system.

CEREBROSPINAL AND OCULAR FLUIDS IN EXPERIMENTAL JAUNDICE IN DOGS*

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Recent studies on the cerebrospinal fluid of infants,[1] indicating the frequency of xanthochromia, have stimulated further investigation of the relationship of xanthochromia in the spinal fluid to clinical jaundice. It occurred to us to determine whether xanthochromia could be produced or bilirubin caused to appear in the spinal fluid in experimental jaundice in dogs. We also wished to determine if the degree of jaundice had any relationship to the xanthochromia that might occur in the cerebrospinal fluid. Further, we desired to learn whether nuclear (kern) icterus,[2] i.e., a yellow staining of variable intensity of certain of the basal ganglions, such as occurs in some cases of icterus neonatorum, could be produced experimentally. The exact nature of the pigment in the nerve cells in this condition has not been shown. It is generally assumed that the pigment is biliary in origin, but the possibility that it is a lipochrome has been suggested.[3] In most of our experiments, we also observed the color of the ocular fluids.

TECHNIC

In the course of the work, both adult and young dogs were used. The adult dogs were divided into groups 1 and 2. To those of group 1, toluylenediamine was given. The dogs were killed a number of days later, and the relation between the intensity of the jaundice in the blood serum and the color of the spinal and

* Submitted for publication, Dec. 9, 1929.

* From the Otto Baer Fund for Clinical Research of the Michael Reese Hospital and the Nelson Morris Institute for Medical Research, Chicago.

1. Roberts, M. H.: Spinal Fluid in the New-Born, with Especial Reference to Intracranial Hemorrhage, J. A. M. A. **85**:500 (Aug. 15) 1925; Intracranial Hemorrhage in the New-Born, South. M. J. **20**:642, 1927; Relation of Pigment Content in Serum and Spinal Fluid of New-Born Infants, ibid. **21**:460, 1928. Levinson, A.; Greengard, J., and Lifvendahl, R.: Cerebrospinal Fluid in the New-Born, Am. J. Dis. Child. **32**:208 (Aug.) 1926.

2. Schmorl, G.: Zur Kenntnis des Icterus Neonatorum, insbesondere der dabei vorkommenden Hirnveränderung, Verhandl. d. deutsch. Gesellsch. **6**:109, 1903. For a discussion in English and other references to this subject see reference in footnote 10.

3. Personal communication to the authors from Dr. G. H. Whipple, of the University of Rochester Medical School.

ocular fluids was observed. The use of toluylenediamine for the purpose of producing jaundice was suggested principally by the work of Eppinger.[4]

In these experiments, it was noted that the greatest degree of icterus occurred from two to four days after the administration of the toluylenediamine, and for this reason the spinal and ocular fluids were usually studied at this time.

In group 2 of the adult dogs, the common duct was first ligated and cut between the ligatures, and then, after a varying number of days, the toluylenediamine was administered as in the case of the dogs of group 1, and the same procedures followed. In one of these dogs, the cystic duct also was ligated.

The young dogs ranged in age from 6 to 8 weeks and were divided into three groups. In group 1, the common duct was ligated and cut between the ligatures, and after a number of days the relationship between the icterus index of the serum and the color of the spinal and ocular fluids was observed. To group 2, toluylenediamine only was administered. These dogs were killed a varying number of days later and studied as was group 1. To group 3, toluylenediamine was given a week after the common ducts were tied and cut. These were studied in the same manner.

The toluylenediamine was administered by stomach tube in the adult and by catheter in the young dogs. The dosages are recorded in tables 1 and 2. In the adult dogs, the drug was dissolved in about 30 cc. of 70 per cent alcohol and then diluted to 150 cc. with water. In the young dogs, the toluylenediamine was dissolved in 10 cc. of 70 per cent alcohol and diluted with water to 60 cc.

The blood was drawn with a perfectly dry syringe and needle from a leg vein, in the case of the adult dogs, and by cardiac puncture in the case of the young dogs. The blood was centrifugated before clotting occurred, and the clot was then loosened and the blood again centrifugated. Clear, nonhemolyzed serum was always obtained. The icterus index in the case of the lighter-colored serums and of the cerebrospinal and ocular fluids was determined by the method of Brown.[5] In the case of the deeply jaundiced serums, the icterus index was determined by comparing the color of the serum in a known dilution with a physiologic solution of sodium chloride with 1:10,000 potassium dichromate in aqueous solution as a standard in a colorimeter. This standard solution has an icterus index of one.[6] The direct and indirect qualitative van den Bergh tests were made by the usual methods in all the serums and fluids.[7] The spinal and ocular fluids were also tested for hemoglobin by the benzidine tablet method of Roberts.[8] The dogs were killed by ether, and the spinal fluid was obtained by exposing the atlanto-occipital ligament, which was punctured by a 22-gage needle. This method

4. Eppinger, H., and Ranzi, E.: Die Hepato-Lienalen Erkrankerungen, Enzyklopadie der klinischen Medizin, Berlin, Julius Springer, 1920. Landa, E., and Starlinger, W.: Ueber den Einfluss der Toluylendiamine, Ztschr. f. d. ges. exper. Med. **51**:213, 1926.

5. Brown, A. L.: A Rapid Clinical Method for the Determination of the Icterus Index, Arch. Path. **3**:309 (March) 1927.

6. Maue, H. F.: Icterus Index of the Blood Serum, Surg. Gynec. Obst. **34**:752, 1922.

7. McNee, J. W.: Jaundice: A Review of Recent Work, Quart. J. Med. **16**:390, 1923. See footnote 10 for technic of van den Bergh tests on spinal fluid. The same technic was also used for the ocular fluids.

8. Roberts, D.: A Benzidin Tablet Test for Occult Blood, J. A. M. A. **65**:244 (July 17) 1915.

ensured the obtaining of fluid free from traumatic blood. The aqueous humor was obtained by aspirating the anterior chamber of the eye with a 27-gage needle.

RESULTS

The results of the experiments are summarized in the accompanying tables. The icterus index of the blood serum of normal adult and young dogs is not stated in either of the tables. It was usually less than 1 and often 0, though it occasionally ranged between 1 and 2 in the older dogs. An icterus index of even 1 in dogs' serum is high,

TABLE 1.—*Results of Experiment to Produce Xanthochromia in Adult Dogs*

	Gm. Toluylene-diamine, per Kg.	Days Between Dosage with Toluylene-diamine and Withdrawal of Fluids	Icterus Index			Van den Aqueous Humor
			Blood Serum**	Spinal Fluid¶	Aqueous Humor	
Group 1*						
Dog 1	0.03	4	15	0	§	§
Dog 2	0.04	10	80	0	§	§
Dog 3	0.10	3	169	0	§	§
Dog 4	0.10	2	182	0	0	0
Dog 5	0.10	4	(Dog dead)	1—†	0	0
Dog 6	0.05	3	182	§	§	§
		4	45	0	0	0
Dog 7	0.08	4	252	1—	1—	0
Group 2*						
Dog 8	0.08	3	370	1—	‡	0
Dog 9	0.05	5	150	1—	3	+
Dog 10	0.08	2	(Dog dead)	0	0	0
Dog 11	0.08	4	180	1—	4	+

* In group 1, the dogs received only toluylenediamine. In group 2, the dogs received toluylenediamine from three to five days after the common bile duct was tied and cut between the ligatures. The integrity of the ligatures was always checked by autopsy. In dog 11, the cystic duct was also ligated.

** Van den Bergh reaction positive in every case.

¶ Van den Bergh reaction negative in every case.

† 1—indicates icterus index less than 1.

‡ This fluid was yellow, but the quantity obtained was insufficient for an accurate determination of the icterus index.

§ Not examined.

since dogs' serum is usually stated to be practically colorless.[9] Perhaps, the slight coloring of the serum in some of our dogs was due to some food factor, as the dogs had not been caused to fast before the beginning of the experiments and were on a mixed diet of milk, meat, bread and vegetables. The van den Bergh reactions of the normal serums were always negative. In group 1 of the adult dogs, the lowest icterus index of the blood serum at the time of obtaining spinal fluid was 15 and the highest 252. The spinal fluid was colorless in six cases; in two, it had a slight yellowish tinge. In one of the latter dogs, the icterus index of the blood serum was 252; the other dog was found

9. Van den Bergh, A. A. H., and Snapper, J.: Die Farbstoff des Blutserums, Arch. f. klin. Med. **110**:540, 1913.

dead, and thus the icterus index of the blood serum could not be determined. The test for hemoglobin was negative in all the spinal fluids. The ocular humors were studied in four of these dogs, and there was a yellow tinge in the aqueous humor only in the dog the serum icterus index of which reached 252. Tests for hemoglobin made on the ocular fluids were negative. The van den Bergh reactions were negative in all the spinal and ocular fluids.

Whenever the van den Bergh test was positive in this work, both direct and indirect reactions were obtained, and thus throughout this paper no further reference is made as to whether the direct or indirect

TABLE 2.—*Results of Experiment to Produce Xanthochromia in Young Dogs*

	Total Dose of Toluylene-diamine, Mg.	Days Between Dosage with Toluylene-diamine, and Withdrawal of Fluids	Icterus Index		
			Blood Serum†	Spinal Fluid†	Aqueous Humor†
		Group 1*			
Dog 1	0	..	(Dog dead)	0	0
Dog 2	0	..	10	0	0
Dog 3	0	..	15	0	0
Dog 4	0	..	8	0	0
		Group 2*			
Dog 5	40	4	8	0	0
Dog 6	40	3	8	0	0
Dog 7	20	2	28	1—	1—
Dog 8	40	3	9	0	0
Dog 9	60	2	12	0	0
		Group 3*			
Dog 10	40	2	15	0	0
Dog 11	40	5	(Dog dead)	0	0
Dog 12	40	2	(Dog dead)	0	0

* In group 1, the common bile duct was ligated and cut between the ligatures. No toluylenediamine was given. The blood, spinal and ocular fluids were examined from eighteen to twenty days after operation. In group 2, the dogs received only toluylenediamine. In group 3, toluylenediamine was given seven days after the common bile duct was ligated and cut between the ligatures.

† The van den Bergh test was positive in all the blood serums and negative in all the spinal and ocular fluids.

test was performed. The same holds true for negative van den Bergh tests.

In group 2 of the adult dogs, the highest icterus index of the blood serum was 370 and the lowest 150 at the time the blood was drawn. In the dogs of this group, a yellow tinge was present in the spinal fluid and aqueous humor in three instances. All these fluids failed to give a hemoglobin reaction. A positive van den Bergh test was obtained in two of the xanthochromatic aqueous humors, but in none of the spinal fluids. The icterus index of the yellow-tinged spinal fluid never even reached 1, but was as high as 3 and 4 in the aqueous humors which gave positive van den Bergh reactions. The vitreous was colorless in all cases.

In group 1 of the young dogs, the highest icterus index of the blood serum observed was 15, and no change was present in the spinal and ocular fluids. In group 3, the highest icterus index of the blood serum was also 15 and again the spinal and ocular fluids remained colorless. In group 2, the icterus index of the blood serum reached 28 in one dog, and in this case a yellowish tinge was present in both the spinal fluid and aqueous humor. Hemoglobin and van den Bergh tests were negative. No change occurred in the spinal and ocular fluids of the other young dogs of this group.

In the case of both adult and young dogs, complete autopsies were performed. Nuclear (kern) icterus did not occur in any instance.

COMMENT

The literature relating to the subject of icterus and xanthochromia in the spinal fluid has been reviewed by Glaser.[10] From it conflicting evidence is noted. Some investigators assert that bile pigments are present in all cases in the spinal fluid in jaundice. Others have found it in only a few cases; still others only in long-standing and severe jaundice. A few found no xanthochromia even in long-standing and severe jaundice. The icteric cases studied were usually of the obstructive type of jaundice with varying etiology. The ocular fluids were not mentioned in these papers.

In the work here reported there was a slight yellow tinge to the spinal fluid in five of the eleven adult dogs. The benzidine test for hemoglobin and the van den Bergh reaction for bile pigment were negative in every instance. The lowest icterus index of the blood serum in which there was an accompanying yellow cerebrospinal fluid was 150. The icterus index of the blood serum ranged as high as 182 without xanthochromia of the spinal fluid. This, perhaps, suggests a threshold for the appearance of xanthochromia under the conditions of our experiments. This is particularly probable since, with icterus indexes this high, owing to the difficulty of exactly matching yellow colors, a difference of 30 points may be within the limits of experimental error. The same general facts hold true for the aqueous humor, but with this fluid the xanthochromia became more intense than in the case of the spinal fluid and the van den Bergh reactions were positive. The vitreous humor remained colorless in all experiments.

The yellow aqueous humor occurring in these experiments suggests a possible explanation of xanthopsia in some cases of jaundice.

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10. Glaser, J.: The Cerebrospinal Fluid of Premature Infants, with Special Reference to Intracranial Hemorrhage and Pigmentation, Am. J. Dis. Child. **36:** 195 (Aug.) 1928.

We were unable to produce any marked icterus in the young dogs comparable with that in the adult dogs. Toluylenediamine seemed to produce a severe anemia in the former without producing an intense jaundice. In one case in which the icterus index of the blood serum reached 28, there was a definite yellow tinge to both the spinal fluid and the aqueous humors, but the van den Bergh reactions in both fluids were negative.

The xanthochromia occurring in the spinal fluid of our dogs in the presence of negative van den Bergh reactions is probably due to the presence of bile pigment in insufficient amounts to give such a reaction but sufficient to produce a faint yellow color. To determine whether this might be possible, we diluted some icteric dog serum with physiologic solution of sodium chloride until we reached a point at which a faint yellow color persisted, but the van den Bergh reaction was negative. The concentration of bilirubin in this solution was 1:1,650,000, if one considers that an icterus index of 100 roughly matches 100 mg. of pure bilirubin per liter.[11] This dilution is thus just beyond the 1:1,500,000 dilution of pure bilirubin in which a positive van den Bergh reaction is still obtained.[12]

CONCLUSIONS

Xanthochromia may be produced in the cerebrospinal fluid and aqueous humor in experimental jaundice in dogs. Its appearance probably depends on the intensity of the icterus of the blood serum as measured quantitatively by the icterus index. Nuclear (kern) icterus was not produced.

In our experiments, the icterus index of the xanthochromatic spinal fluids never exceeded 1; the icterus index of the yellow aqueous humors ranged as high as 4. The vitreous humor always remained colorless.

The van den Bergh reactions were always negative in the xanthochromatic spinal fluids, but positive in the aqueous humors which had an icterus index of 3 or 4.

233 Oxford Street.

11. Cutten, C.; Emerson, E. E., and Woodruff, W.: The Icterus Index, Arch. Int. Med. **41**:428 (March) 1928.

12. Van den Bergh, A. A. H.: Der Gallenfarbstoff im Blute, Leyden, S. C. van Doesburgh, 1918.

General Review

THE EVIDENCE FURNISHED BY BIOCHEMISTRY AND IMMUNOLOGY ON BIOLOGIC EVOLUTION*

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The story of the evolution of existing forms of life has been reconstructed almost entirely from anatomic evidence; of necessity, in respect to the evidence that has come from the fossils, and by tradition and inclination with most of the rest of it. In 1859, when Darwin put the subject of evolution on the front page of the daily papers and on all the pages of the religious journals, biologic science was mostly a matter of morphology and classification, as befits a young science. It was only in 1828 that Wöhler had shattered the prevalent idea that organic substances must be the product of vital processes, simply by producing urea from ammonium cyanate. Pepsin had been discovered by Schwann only in 1836, and the digestive function of the pancreas had been revealed by Claude Bernard in 1846. These discoveries, together with that of the glycogenic function of the liver by Bernard, and Helmholtz' recognition of the muscles as the source of animal heat in 1848, had been large factors in giving rise to biologic chemistry as a separate division of physiology. Not until 1864 did Graham point out the fundamentals of colloidal chemistry. Organic chemistry had newly received its great stimulus in the production of aniline dyes from coal tar by Perkins in 1856. The "Cellular Pathology" of Virchow was only a puling infant of a few months' life. It was six years before Gregor Mendel announced the laws of heredity in a publication that was to be lost to the world until the same laws were rediscovered thirty-five years later. Physiology had not become sufficiently developed as a distinct field of science for the establishment of a Physiological Society of London until 1875, four years after Darwin's second great contribution, The Descent of Man, had been published.

Although evolution had been discussed, debated, and to some extent investigated even from "the Greeks to Darwin," as Osborn pointed out,

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* From the Department of Pathology of the University of Chicago and the Otho S. A. Sprague Memorial Institute.

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its birth as a major consideration in human thought was in 1859, in a cradle of science constructed almost solely of the branches of morphology, with coverings of systematization. Necessarily, the considerations of evolution in its early years were based on the evidence then available, which was almost exclusively structural, and because of this sort of environment in infancy its subsequent career has largely depended on morphologic contributions and considerations. This asymmetrical development is so marked that, as recently as 1926, Leathes in his presidential address to the Section on Physiology of the British Medical Association complained, and asked, "Why is it that what may be termed official physiology takes so little cognizance of the doctrine of evolution?" He suggested that possibly it was because these branches of biologic study followed courses so exactly parallel that they never met. And he pointed out that "natural selection applies to the survival of the chemical forms of living matter as it does to complex living organisms. These forms, infinitely protean in their variety, survive and persist in so far and so long as they minister to self-regeneration. It is the principle of survival by service. Function alone gives permanence to structure." Therefore it is essential that function, with all that this involves, receive full consideration in working out the course of the evolution of the living world of today.

THE ORIGIN OF LIVING MATTER

Now, biologic function certainly depends even more on chemical properties than on anatomic organization. Formless enzymes may function actively, whereas the most elaborate anatomic structures in our museum jars function not at all, because their enzymes are dead. The evolution of life presumably had first to be the evolution of proteins, carbohydrates and fats, which then somehow constituted the living, probably at first formless, matter, and these were the important first steps in evolution. All the later strange divergencies into such elaborate creatures as cabbages and kings are matters of minor importance.

Therefore one turns to chemistry to see if it can offer a solution of the problem of the origin of life. Such explanations of the origin of life on the earth as that it is the result of the transportation of spores by meteors or star dust, even if true, are no solution, since they merely push back the origin of life to some other part of the universe. Presumably, one may suspect that the origin of life was the sequel of the formation of inorganic colloids, which served as the antecedents of the formation of organic colloids. It is generally agreed that life depends on the existence of organic colloids, partly because they form peculiarly labile compounds with crystalloids and water and perhaps

partly because, as I have elsewhere suggested,[1] colloids interfere greatly with the diffusion of other colloids and but little with the diffusion of crystalloids.

Moore[2] speculated interestingly from the point of view of the biochemist on this matter of how life came to be. Recognizing the colloidal basis of life, he started it with the development of inorganic colloids, which could begin to form by aggregation of inorganic molecules when the materials of the primitive world had reached a temperature that permits such aggregates to exist.

Moore said:

> The first primeval step would appear to be indicated by the union of single crystalloidal inorganic molecules to form inorganic colloids, and these metastable colloids acting on inorganic carbon compounds, such as carbon dioxide, in the presence of water and sunlight, and taking energy from the sunlight, built up at first simple organic bodies, and now these in turn reacting with one another formed more and more complex organic compounds. The fact that the present basis of the system of living creatures in the world is light energy leads to the view at a certain stage in the development of colloids, probably long before the appearance of chlorophyll, the colloids began to be affected by the light, and acquired the property of retaining and utilizing light energy for further development of structure, or, in other words, synthesis of more complex colloids. As the complexity of structure increased, the nature of the equilibrium in the colloidal aggregates would approximate more and more to that labile, easily destroyed, but also readily constructive condition which has been described as characteristic of all living things. In this manner we can conceive that the gulf between non-living and living things can be bridged over, and there awakens in our minds the novel conception of a kind of spontaneous generation of a different order from the old. The territory of this spontaneous production of life lies not at the level of bacteria or animalculae springing forth into life in dead organic matter, but at a level of life lying deeper than anything the microscope can reveal, and possessing a lower unit than the living cell, as we form our concept of it from the tissues of higher animals and plants.

The evidence of geology clearly shows that life in some form appeared promptly when conditions of temperature made it possible, since the oldest sedimentary rocks show that already there were living forms that had much to do with the formation of these early strata of the world's crust. In the earliest of all stratified rocks, in the pre-Cambrian deposits of the Canadian Rockies, Walcott found fossilized structures that he interpreted as probably algae and bacteria, which were presumably an important factor in the deposition of these limestones before coralline structures had appeared in the world.

Moore reported that his experiments with a view to testing the synthetic action of sunlight in the presence of inorganic colloids (silicic

1. Wells, H. Gideon: Chemical Pathology, ed. 2, Philadelphia, W. B. Saunders Company, 1914, p. 33.

2. Moore, Benjamin: Biochemistry, a Study of the Origin, Reactions and Equilibria of Living Matter, London, Longmans, Green & Company, 1921.

acid or colloidal uranium oxide) had shown it possible to observe the synthesis from CO_2 and H_2O, of formaldehyde (CH_2O), which is supposed to be the first step in the formation of carbohydrates, which are merely multiples of formaldehyde, e. g., $6CH_2O = C_6H_{12}O_6$. Indeed, under the influence of light, formaldehyde polymerizes to produce substances which reduce copper solutions as do the carbohydrates. It is further possible for this formaldehyde in the presence of nitrates or nitrites to produce substances that can build up to form amino acids, and hence the fundamental steps in the evolution of carbohydrates and proteins are possible in the absence of living precursors—only carbon dioxide, water, nitrites and sunlight are necessary, and inorganic colloids are capable of speeding up the process.

It is interesting to note how close Lamarck came to this idea of the origin of life, long before there was any knowledge of colloidal chemistry, for in 1802 he wrote:

> In the waters of the ancient world, and at the present time, very small masses of mucilaginous matter were collected. Under the influences of light, certain elements, caloric and electric, entered these little bodies. These corpuscles became capable of taking in and exhaling gases; vital movements began, and thus an elemental plant or animal sprang into existence. Possibly higher forms of life, such as infest the intestines, originate in this way. Nature is thus always creating.[3]

If, as Moore suggested, life began by the development of formless colloidal complexes capable of accelerating synthesis under the influence of light rays, there still remains an unexplained wide gap between such hypothetical chemically active colloidal masses and the simplest microscopically visible living forms, the bacteria. Possibly, the invisible, filtrable viruses represent an intermediate stage antecedent to the bacteria, but there is no knowledge as to how much real structure viruses have, or how many sorts there may be which cannot be recognized, because only those that produce disease make themselves known. It has even been suggested that the bacteriophages may be something like the genes that determine hereditary transmission of living characters, since they seem to be of approximately the same magnitude, that is, close to 50 millimicrons in diameter.[4]

While such conceptions are at present purely speculative, they have enough reason in them, even with the present scanty knowledge, to make it not fantastic that with more knowledge something like them may be found to be true. It is possible that one lives in a world of unknown, submicroscopic, little-organized living material, but the lack of observable chemical change in nutrient materials from which bacteria have been removed by filtration is against such a hypothesis.

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3. Lamarck, quoted by Osborn: "From the Greeks to Darwin," New York, The Macmillan Company, 1908.

4. Alexander and Bridges: Science **70**:1821, 1929.

Presumably, the next stage in the evolution of life lies in those bacteria which can grow on inorganic mediums and synthesize their proteins, lipoids and carbohydrates from carbon dioxide and inorganic nitrogen, sulphur and phosphorus compounds. They secure their energy by oxidation of inorganic compounds, such as those containing the sulphydryl and ferrous iron, and do not require organic compounds as a source of energy; therefore they can live and produce their own organic compounds without the aid of chlorophyll. And what seem to be fossil bacteria in the pre-Cambrian limestones have been described by Walcott.

The step from such synthesizing bacteria to the algae with chlorophyll was probably the next important phase in the actual creation of life as one now knows it, for only with the coming of chlorophyll to utilize the sun's energy did life on a large scale become possible. Hence the study of the nature and origin of chlorophyll may well give much information as to the steps by which the present living world was evolved. It certainly is of great significance that the oldest known geologic deposits that contain traces of living forms exhibit structures that seem to be algae, since even now the lowliest known organisms capable of synthesizing organic substances are the chlorophyll-containing algae. The bacteria with synthetic powers which can build up from inorganic materials their complex proteins and enzymes with all the properties of life, presumably preceded and evolved into the algae, but they could thrive only in the absence of direct sunlight which is fatal to them. Chlorophyll not only augments synthetic function, but by filtering off the ultraviolet rays enables life to go on despite the destructive action of sunlight.

THE EVOLUTION OF PROTEINS

Since the colloids characteristic of life have as their basic element the proteins, one of the most important of the first steps in evolution was the evolution of the amino acids that make up the proteins. Disregarding as not essential for the present the question of how the amino acids are linked together,[5] one finds that in some mysterious way only a remarkably small number of the exceedingly great number of theoretically possible amino acids have been utilized to form the proteins. Only the amino acids with the amino group in the alpha position, next to the carboxyl group, seem to have been utilized, and of the vast number of possible alpha amino acids, only about a score have been needed to form all the kinds of proteins that go to make up the living world. Those most generally found are given in table 1.

5. Vickery and Osborne: A Review of Hypotheses of the Structure of Proteins, Physiol. Rev. **8**:393, 1928.

TABLE 1.—*Amino Acids Used in the Formation of the Proteins**

I. MONO-AMINO-MONOCARBOXYLIC ACIDS

1. Glycine ..... $CH_2NH_2.COOH$
2. Alanine ..... $CH_3.CHNH_2.COOH$
3. Valine ..... $\begin{matrix}CH_3\\CH_3\end{matrix}\!>\!CH.CHNH_2.COOH$
4. Leucine ..... $\begin{matrix}CH_3\\CH_3\end{matrix}\!>\!CH.CH_2.CHNH_2.COOH$
5. Isoleucine ..... $\begin{matrix}CH_3\\CH_3CH_2\end{matrix}\!>\!CH.CHNH_2.COOH$

II. HYDROXY-MONOAMINO-MONOCARBOXYLIC ACID

6. Serine ..... $CH_2OH.CHNH_2.COOH$

III. DIAMINO-DICARBOXYLIC ACID CONTAINING SULPHUR

7. Cystine ..... $HOOC.CHNH_2.CH_2—S—S—CH_2.CHNH_2.COOH$

IV. MONO-AMINO-DICARBOXYLIC ACIDS

8. Aspartic acid..... $HOOC.CH_2.CHNH_2.COOH$
9. Glutamic acid..... $HOOC.CH_2.CH_2.CHNH_2.COOH$

V. DIAMINO-MONOCARBOXYLIC ACID

10. Lysine ..... $NH_2.CH_2.CH_2.CH_2.CH_2.CHNH_2.COOH$

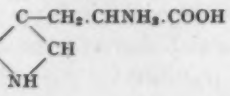
VI. DIAMINO-MONOCARBOXYLIC ACID WITH GUANIDINE GROUP

11. Arginine ..... $HN=C\!<\!\begin{matrix}NH_2\\NH.CH_2.CH_2.CH_2CHNH_2.COOH\end{matrix}$

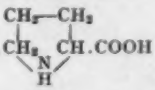
VII. WITH BENZENE RING

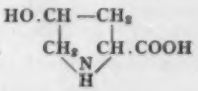
12. Phenylalanine ..... $C_6H_5.CH_2.CHNH_2.COOH$
13. Tyrosine ..... $HO.C_6H_4.CH_2.CHNH_2.COOH$

VIII. WITH INDOLE RING

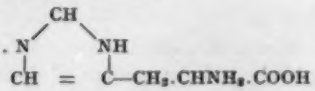
14. Tryptophan ..... 

IX. WITH PYRROLE RING

15. Proline ..... 

16. Oxyproline ..... 

X. WITH IMIDAZOLE RING

17. Histidine ..... 

* From Kennaway: Notes on the Evolution of Proteins, Chem. News **13**: 120, 1920.

On scanning the list in table 1, one sees at once that these nowhere form a complete series. For example, if there is one hydroxy acid (serine) corresponding to alanine, why should there not be found the hydroxy derivatives of all the other amino acids? And why but one sulphur-containing amino acid, and but two dicarboxylic acids, and three diamino acids, and three with the benzene ring, when apparently it would be just as easy to form many more? Presumably, vastly more than these few sorts of amino acids have occurred in nature, yet for some reason they have been discarded as unsuitable or unnecessary for the sorts of proteins that are required to make a living organism. Nor are all the few known amino acids of proteins necessary for each and every protein, for no protein yet analyzed has been found to contain all of them, and many proteins seem to contain relatively few. It is perhaps significant that the proteins most concerned with cell multiplication and heredity, the nucleoproteins of the germ cells, seem to have the smallest number of different amino acids, as if these few amino acids were the essential ones to keep life going and reproducing, while the rest represent merely those responsible for the differentiation and the functions less essential than reproduction.

The possible number of isomeres of an organic compound depends on the number of asymmetrical carbon atoms; hence proteins, formed of linked amino acids, most of which contain such asymmetrical carbon atoms, possess exceptional possibilities for chemical differentiation. As Lillie [6] emphasized, "The specific characters of any animal or plant are determined ultimately by the specific characters of its structure-forming proteins. The developing germ or growing organism synthesizes specific proteins, and these, since they determine the structural and hence the physiological peculiarities of the organism, form the basis of its special character as an organic species."

As to the possibilities for variation present in the composition of proteins made up of such a limited number of amino acids to account for the vast numbers of known and unknown, living and extinct forms of life, this statement may be taken from Leathes:

> Supposing it were a chain of only fifty links, a very simple case; if all the links were different the number of possible permutations is denoted by the innocent-looking symbol $\underline{|50}$. If, instead of all being different, one kind of link recurred ten times, the number would be reduced to $\underline{|50}/\underline{|10}$. If, in addition, there were four that recurred four times and ten recurred twice, it would be further reduced to
>
> $$\underline{|50}/\underline{|10} \times (\underline{|4})^4 \times (\underline{|2})^{10}.$$
>
> It would now consist of a chain of only fifty links, of which there were only nineteen different kinds, and the number of different arrangements of its parts would

6. Lillie, Ralph: Biol. Bull. **34**:65, 1918.

be about 10^{48}. Astronomy deals with big figures. Light, it is said, takes 300,000 years to travel from one end of the Milky Way to the other; this distance expressed in Angstrom units, 10,000,000 of which go to a millimeter, would be less than 10^{32}. So far are we from knowing the structure of protein molecules. So far are we from knowing what variations in disposition of the parts in such a molecule may not occur without our being within a measurable distance of detecting them. For if the number of possible varieties of a protein whose molecular weight is known, and known to be exceptionally small, and which contains the several amino acids in a known proportion, is as great as this, the number that is possible when that proportion may be changed is practically incalculable, each change in proportion being capable of a number of new arrangements that could be calculated, as was done for our hypothetical case.

It is most significant, furthermore, that much the same amino acids are found in all living cells, whether they be simple bacteria and yeasts, or the vastly more complex plants and animals. As Kennaway said of these analytic results:

> They show that the simplest organisms now existing do not contain a series of amino-acids any more primitive than that present in the higher organisms. One may suppose that the present apparently stereotyped series of utilisable amino-acids represents the stable outcome of a struggle long ago among simple organisms in which those which made a less suitable choice were beaten, and have passed away leaving no trace. We cannot know the biochemistry of the first organisms which appeared upon the earth; the experiments and discarded compounds of that time are lost. The selection of amino-acids must have taken place at an immensely remote period, for the earliest records which we have of the forms of life on the earth do not show us organisms which have any appearance of noteworthy difference in chemical composition from those which exist at the present day. Thus one of the earliest fossils, the brachiopod Lingula of the Cambrian period, is very closely related to a species now living. Structures regarded as fossil Algae very similar to recent species have been found in still older rocks. The doctrine of natural selection gives the impression that evolution proceeds throughout in a very gradual manner. But at the time when the amino-acids were first being produced and tested, organic evolution must have proceeded very distinctly *per saltum* as each new compound was synthesized; natural selection would then act slowly and surely upon the organisms which made one or another choice, and thus the present series of amino-acids was delimited.

In view of the general principle that the individual in its development tends to recapitulate the development of its species, one might hope to find the various steps of the evolution of the protein in the substances produced when bacteria or yeasts synthesize their own proteins from the simple mixtures of salts that they are able to utilize in reproducing themselves in vast numbers. But unfortunately this is not the case. The reproduction is completed so rapidly that one cannot catch the different stages. This is indeed a marvelous feat, and Kennaway said:

> In the time taken by a yeast cell to produce another by budding, when growing on a medium such as Pasteur's containing no nitrogen but that of ammonium tartrate, it must synthesise each one of the amino-acids present in the yeast cell,

and combine them as a series of polypeptides until its proteins are produced, and all the while carry on many other chemical operations. Even when one takes into account the small size of molecules it is wonderful that so many reactions can be kept apart within the compass of a yeast cell.

CHEMICAL STEPS IN EVOLUTION

One cannot hope, therefore, to learn through chemical analysis of growing cells the steps by which the proteins as they exist now were evolved. Even chemical study of that beautiful material for the investigation of the evolution of the individual, the developing eggs of hens, has not as yet thrown any light on the problems of evolution, although there is room for much more profitable study in this field.[7] As Needham pointed out, the developing egg is a closed system, without the usual complications for the biochemist of intake, excretion and bacteria. From a small variety of proteins, carbohydrates and fats, are formed in a few days the vast variety of components of the mature chick. From morphologic studies of the developing chick, much has been learned about the evolution of structure. Undoubtedly, closer biochemic study of developing eggs would give evidence as to the evolution of chemical structures. The vitally important nucleic acids, with their purines and pyrimidines which form so large a part of the hatching chick, are almost entirely new-formed from other components during incubation. What are the steps of evolution of purines and nucleic acids? Can one not learn the chemical evolution of keratin, as well as one knows the morphologic evolution of birds' feathers from fishes' scales? If one could follow the transformations that produce hemoglobin in the egg, one would probably learn how it came into existence in the Cambrian. And one might follow the development of enzymes as well. Needham, who is one of the few biochemists attacking the problem of the evolutionary significance of the changes in the developing egg, brought out an interesting bit of evidence of the well known relationships of birds and cold blooded forms by showing that the developing chick and dogfish embryos alike have to synthesize 90 per cent of the scyllitol that they produce.[8] There is, furthermore, the suggestive discussion of the "Paleochemistry of Body Fluids and Tissues" by Macallum[9] suggesting that the inorganic constituents of one's body portray the composition of the pre-Cambrian seas from which one's ancestors arose.

That important functions may not come until late in the development of the egg is well illustrated by the observation of Murphy[10] of the

7. Needham, J.: The Metabolism of the Developing Egg, Physiol. Rev. **5**:1, 1925.

8. Needham: Biochem. J. **23**:319, 1929.

9. Macallum, A. B.: Physiol. Rev. **6**:316, 1926.

10. Murphy: J. Exper. Med. **19**:181, 1914.

Rockefeller Institute, that in the early days of development of the chick it offers no resistance to the implantation of foreign tumor cells, even mammalian tumor cells. Only after the eighteenth or nineteenth day of incubation does the chick acquire the capacity to destroy the foreign cells, which it does suddenly and effectually. Even after that stage of its evolution, the chick is able to prevent mammalian tumor cells from living in its tissues.

Undoubtedly, biochemistry can give more than it so far has to establishing relationships and distinctions between different animal forms, to supplement or to test the evidence that has been furnished by anatomic studies. For example, anatomic studies have shown that the central nervous system is derived from the same cells as those which produce the surface epithelium. In accord with this, biochemists have found that the central nervous system contains a form of keratin, neurokeratin, which seems to be chemically like the keratin which characterizes the surface epithelium. And forty years ago, Kühne and Chittenden [11] showed that such species as lobsters, which have chitin for an exterior covering instead of keratin, also have neurochitin instead of neurokeratin in their nervous tissues.

How much heredity and evolution are matters of chemistry and enzymes is apparent when one considers how much weight one gives to the color of the coat in establishing the relationships of animals, whether one is observing the furs of quadrupeds, the feathers of birds or the skin of man. For these colors mostly depend on the amount of melanin formed from aromatic compounds by the action of oxidizing enzymes, and the characteristically inherited character of albinism depends apparently on an absence of the proper enzyme in some cases, and in others on the presence of a specific inhibitor of the enzyme (Onslow).[12]

EVOLUTION OF THE PURINE ENZYMES

The story of the enzymes of purine metabolism [13] is a beautiful example of the possible unearthing of biochemical evidence in respect to evolutionary history. The steps by which the complex nucleoprotein molecule is broken down by several different specific enzymes is something like this:[14]

(1) Nucleic acid + nuclease → H_3PO_4 + carbohydrate + purine (adenine or guanine).

11. Kühne and Chittenden: Ztschr. f. Biol. **26**:291, 1890.

12. Onslow: Proc. Royal Soc. London (B) **89**:36, 1915. Marinesco: Rev. Sc. **60**:321, 1922.

13. Wells, H. Gideon: Some Features of Purine Metabolism, J. Lab. & Clin. Med. **1**:164, 1915; Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925, chap. 25.

14. For simplicity the various different lines of cleavage in the disintegration of nucleic acid are not considered separately.

These two purines are then attacked by specific deaminizing enzymes, guanase or adenase, as shown in the following matter:

$$\underset{\text{(guanine)}}{C_5H_5N_5O} + H_2O \xrightarrow{\text{guanase}} \underset{\text{(xanthine)}}{C_5H_4N_4O_2} + NH_3$$

$$\underset{\text{(adenine)}}{C_5H_5N_5} + H_2O \xrightarrow{\text{adenase}} \underset{\text{(hypoxanthine)}}{C_5H_4N_4O} + NH_3$$

The hypoxanthine formed from adenine is then oxidized by a specific enzyme to xanthine

$$\underset{\text{(hypoxanthine)}}{C_5H_4N_4O} + O \xrightarrow{\text{(hypoxanthine oxidase)}} \underset{\text{(xanthine)}}{C_5H_4N_4O_2}$$

Then the xanthine which has been derived in this way from both adenine and guanine is oxidized to uric acid

$$\underset{\text{(xanthine)}}{C_5H_4N_4O_2} + O \xrightarrow{\text{(xanthine oxidase)}} \underset{\text{(uric acid)}}{C_5H_4N_4O_3}$$

And lastly, in many species of animals the uric acid is further oxidized to the more soluble allantoin

$$\underset{\text{(uric acid)}}{C_5H_4N_4O_3} + O + H_2O \xrightarrow{\text{(uricase)}} \underset{\text{(allantoin)}}{C_4H_6N_4O_3} + CO_2$$

It is thus evident that the steps of the disintegration of nucleic acid are numerous, but that each separate process is a simple one; and also, that it has been possible to follow out and distinguish the several steps and to establish the fact that each step depends on a distinct and specific enzyme. Not only has all this work been accomplished, especially by Walter Jones and his associates, but, furthermore, these several enzymes have been studied in many different species and tissues, and found to have a most peculiar and characteristic distribution. Not every tissue possesses all the enzymes of purine destruction, and in different species of animals the distribution of the enzymes is different. For example, the enzyme xanthine-oxidase, which oxidizes xanthine into uric acid, is found in man only in the liver, and in other animals it is also of limited distribution, being found usually only in the liver or in the liver and the kidneys, but in the dog it seems to be present in several tissues. The deaminizing enzymes, adenase and guanase, are much more widely distributed, but by no means universally. Adenase, for example, is not present in the tissues of the rat or those of adult human beings. Guanase is absent from the spleen and the liver of the pig and from human spleen, although present in most other tissues. Uricase, the enzyme which destroys uric acid, also has peculiarities of distribution, being seldom found in any tissue other than the liver or the kidney, and being absent entirely from the tissues of man and from the birds and reptiles so far examined. The significance of this distribution of uricase will be discussed at greater length a little later.

Now, while it is unquestionably true that the results of laboratory experiments with tissue extracts cannot always be used literally in the elucidation of metabolism in living animals, yet it has developed that the results thus obtained with purine enzymes fit remarkably well with results of studies of metabolism, so that they are entitled to consideration and confidence. For example, the absence of uricase in man, birds and reptiles agrees perfectly with the fact that in the same animals one finds much excretion of unchanged uric acid, whereas in all those animals that excrete little or no uric acid and much allantoin, we find the enzyme uricase. Furthermore, a striking correspondence is found in the absence of guanase from the liver and the spleen of the pig and the occurrence of a form of gout in pigs with deposition of guanine rather than of uric acid in the tissues.

In connection with this discussion of the biochemical evidence on evolution, there are certain interesting observations on the general biologic features of the purine enzymes. One is that these enzymes appear one by one in the development of the individual, and that the order of their appearance is correlated, in general, with their distribution in the ascending scale of animal evolution. To illustrate—in the developing pig it was found by Mendel and Mitchell that only nuclease is present in the earliest stages, adenase appears by the time the fetus has reached a length of 50 mm., but xanthine oxidase appears only at about the time of birth, while uricase appears somewhat later. So, too, in the human fetus, guanase is present by the third month, but xanthine oxidase does not appear until at or near full term, and uricase never develops.

If one compares these observations with the occurrence of these same enzymes in organisms of varying stages of development in the biologic scale one finds a correlation that must be more than fortuitous. Thus, in a unicellular organism (yeast) there are found only nuclease, which seems to be present in all living cells, and guanase; this corresponds to the early human or pig embryo, each with nuclease and one deaminizing enzyme. A mollusk *(Sycotypus canaliculatus)* was found to contain nuclease and both deaminases, corresponding to a later stage of mammalian development. The reptiles and birds are in the same stage of development as the mature human or pig fetus, being equipped with nuclease, adenase, guanase and xanthine oxidase, but unable to destroy uric acid because of a lack of uricase. Uricase seems to be a late development, acquired chiefly by mammals, and not by all of them. On the basis of the facts cited, and supported by others not mentioned, one may say that in the formation of purine enzymes one has an apparent exemplification of the biologic law that the individual in its development tends to recapitulate the development of its species—"ontogeny recapitulates phylogeny."

Our studies [15] on the occurrence of purine enzymes in tumors also gave interesting results in general harmony with the statements made in the preceding paragraphs, for it was found that benign tumors tend to have the full equipment of enzymes of adult tissues, while malignant tumors are likely to lack the enzymes that appear latest, and thus exhibit their embryonic character chemically, just as they do under the microscope.

As pointed out, uricase, the enzyme that oxidizes uric acid to allantoin, is a late development in the mammals, and in them not formed until the time of birth. While present in nearly all mammals so far examined, it is interesting to learn that the chief exception is formed by man and his closest relations, the higher anthropoids. All the common domestic and laboratory animals that have been examined, including a marsupial, the opossum, destroy most of the uric acid formed in their metabolism and excrete it as allantoin. It is always possible to demonstrate the presence of uricase in the tissues of these animals. Even different species of monkeys are likewise endowed with uricase, but not the orang-utans and chimpanzees that we have examined. Hence these two anthropoid apes (and presumably the gorilla, which has not been studied) stand together with man apart from all other animals in their deficiency in uricolytic enzymes. It is of particular interest that we find here that the anthropoids resemble man, while the monkeys resemble the lower mammals, since this observation furnishes a chemical corroboration of other biologic facts indicating that the anthropoid apes are more closely related to man than to the monkeys. And it is a striking fact that in this particular respect man and the anthropoid apes are less highly developed than other mammals, for, as pointed out previously, the uricase is a late development in the animals that possess it, and apparently is the last acquisition in purine metabolism in the course of the development of many lines in the animal kingdom. Presumably, it is this lack of uricase to destroy uric acid that accounts for the existence of gout as a human disease; for birds, also lacking uric acid, readily get gouty deposits, and swine, which lack guanase to destroy guanine, suffer from a form of gout, with deposits of guanine.

As far as purine metabolism is concerned, man and the anthropoid apes rank in development with birds, reptiles and some mammalian fetuses at about full term. But amphibians and some fish (selachians) can oxidize uric acid,[16] although the bony fish (teleosts) cannot do so. The amphibians, indeed, go even farther than the mammals, for they destroy the allantoin.

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15. Wells and Long: Ztschr. f. Krebsforsch. **12**:598, 1913. Long: J. Exper. Med. **18**:512, 1913.

16. Przylecki: Arch. internat. physiol. **26**:33, 1926.

An interesting analogy is observed in respect to the fatty acid metabolism of man and the anthropoids, for, as pointed out by Friedemann,[16a] among the mammals so far investigated, only man and the apes seem to develop ketosis with excretion of aceto-acetic and β-hydroxybutyric acid on fasting. Apparently, the other mammals possess some sort of ketolytic mechanism not possessed by man and the anthropoid apes, whereby they are protected from the ketosis from which man and the apes often suffer.

In these respects, as well as many others, man is far from being the most highly developed of the mammals. One is too accustomed to assume complacently that man represents the apex and consummation of evolution. As a matter of fact, he occupies in many respects merely an intermediate position among the mammals, and excels only because of the superior development of his central nervous system, much aided by the lack of specialization of his upper extremity. Newman said that this is among the most primitive limbs of any of the land mammals. It is not modified for any single function, but remains generalized and therefore a versatile tool of the brain. Undoubtedly, man would be much better off and immune to gout if he had acquired the power possessed by nearly all other mammals, of converting uric acid into the vastly more soluble allantoin. And he must stand humbled before the more talented frog, which can also convert the allantoin into urea. It is safe to assume that frogs do not have gout. Needham[17] evolved a most entertaining theory of why birds and reptiles excrete uric acid, which time and space will not permit me to discuss as fully as I am tempted to do. Briefly, the idea is that in terrestrial oviparous animals the amount of water available for the excretion of the nitrogenous waste of the embryo into the closed system formed by the egg shell is so limited that only a precipitable nitrogenous substance, such as uric acid, can be gotten rid of without its diffusing back into the embryo. The chick embryo possesses uricase up to the sixth day, and in that period excretes urea and ammonia, recapitulating its aquatic ancestry chemically as it does anatomically with its gill clefts. "When the reptiles came ashore they found it was as well to leave their uricase behind them." But as uric acid is an extravagant excretion product, wasting more carbon and oxygen than urea and ammonia, it seems to have been utilized chiefly by those species in which it serves a purpose in the developmental stages—man and the anthropoids being an unexplained exception.

There is a remarkable, almost amusing, exception to the statement that all mammals except man and the higher apes oxidize uric acid to allantoin before excreting it, for the exception is the black and white

16a. Friedemann: Science **67**:221, 1928.

17. Needham, J.: Science Progress **23**:633, 1929.

spotted coach hound, the dalmatian, that used to trot behind those vehicles that were "classy" enough to be called equipages. It has become almost extinct since gasoline superseded oats as a motive power. Benedict stumbled on the fact that unlike every other sort of dog, pedigreed or mongrel, that any one has examined, the dalmatian excretes large amounts of uric acid, but also much more allantoin than does man. The latter fact indicates that it is not entirely devoid of uricolytic power, and the liver of one dalmatian that I studied showed the presence of uricase.[18]

Breeding of dalmatians with other dogs has produced hybrids which Onslow[19] found to show a segregation of the character involving the power to destroy uric acid from the character involving lack of this property. In the first hybrid generation, the excretion of uric acid was low and that of allantoin high, as in normal dogs, but in the second hybrid generation one of five dogs excreted much uric acid, as does its dalmatian grandparent. Evidently, then, this reduced ability of the dalmatian coach hound to oxidize uric acid is a recessive character. Perhaps this may be looked on as a biochemical illustration of a genetic mutation.

This story of the stepwise development of the enzymes of purine metabolism fits well with the hypothesis that ontogenetic evolution may depend on a series of enzymes within the cells which become active in fixed succession and thus determine the morphologic and physiologic properties gradually manifested in the development.[20] This and related lines of thought have become the basis of systematic investigations, especially by Goldschmidt,[21] and von Euler and his associates,[22] who report that they have shown the dependence of certain mendelian manifestations of heredity in barley on the distribution of enzymes in the chromosomes.

HEMOGLOBIN CRYSTALS AS EVIDENCE OF EVOLUTIONARY RELATIONSHIP

It is apparent that the contributions of biochemistry to the story of evolution are waiting on more knowledge and better methods, for one finds that the most suggestive items have been provided by the

18. Wells, H. Gideon: The Purine Metabolism of the Dalmatian Coach Hound, J. Biol. Chem. **35**:221, 1918.

19. Onslow: Biochem. J. **17**:334 and 564, 1923.

20. Beijerinck: Proc. koninkl. Akad. Wetenschappen, Amsterdam **19**:1275, 1917.

21. Goldschmidt, R.: Quart. Rev. Biol. **3**:307, 1928. Guyer: Science **71**:169, 1930.

22. Von Euler, H.; Hellström, H., and Runehjelm, D.: Ztschr. f. physiol. Chem. **182**:205, 1929; **185**:74, 1929.

use of the most delicate methods for differentiation of proteins—crystallography and immunology. Unfortunately for biochemistry, the crystallization of proteins is difficult, and with most of them impossible. The studies of Reichert and Brown [23] on proteins that do crystallize, the hemoglobins, show what can be done by the exact methods of crystallography. These disclose that even with such a substance as hemoglobin, which, having a common function in all species, might be expected to be much the same in all species, recognizable differences do occur. Although the constant recurrence of certain angles in the hemoglobins of various species, even when the species are widely separated and the crystals belong to various crystal systems, indicates a common structure of the hemoglobin molecules, whatever their source, nevertheless, constant differences are present which distinguish one species from another.

For example, Reichert [24] said:

> When orders, families, genera or species are well separated the hemoglobins are correspondingly markedly differentiated. For instance, so different are the hemoglobins of *Aves, Marsupialia, Ungulata* and *Rodentia* that there would be no more likelihood of confounding the hemoglobins than there would be of mistaking the animals themselves. Even where there is much less zoological separation, as in the case of the genera of a given family, but where there is well-marked zoological distinction, the hemoglobins are so different as to permit readily of positive diagnosis. When, however, the relationships are close the hemoglobins are correspondingly close, so that in instances of an alliance such as in *Canis, Vulpes* and *Urocyon,* which genera years ago were included in one genus (and doubtless correctly) the hemoglobins are very much alike, and in these cases they may exhibit closer resemblances than may be found in general in specimens obtained from well-separated species of a genus.
>
> So distinctive zoologically are these modified forms of hemoglobins that we had no difficulty in recognizing that the common white rat is the albino of *Mus norvegicus* (*Mus norvegicu albus* Hatai) and not of *Mus rattus,* as almost universally stated, and that Urisidae are related to Phocidae (as suggested by Mivart thirty years ago), but not to Canidae, as stated in modern works on zoology. Moreover, we were quick to detect errors in labeling, as, for instance, when a specimen marked as coming from a species of *Papio* was found to belong to one of the *Felidae.* Generic forms of hemoglobin when obtained from well-separated genera are, in fact, so different in their molecular structures that when any two are together in solution they do not fuse to form a single kind of hemoglobin or a homogeneous solution, but continue as discrete disunited particles, so that when crystallization occurs each crystallizes independently of the other and without modification other than that which is dependent upon such incidental conditions as are to be taken into account ordinarily during crystallization. Thus, the hemoglobin of the dog crystallizes in rhombic prisms which have a diamond-shaped cross-section; that of the guinea-pig in tetrahedra; that of the squirrel in hexagonal plates; and that of the rat in elongated six-sided plates. When any two of these

23. Reichert, E. T., and Brown, A. P.: The Crystallography of Hemoglobins, Publication 116, Carnegie Institution of Washington, D. C., 1909.

24. Reichert: Science **40**:649, 1914.

hemoglobins are together in solution and crystallization occurs, each appears in its own form. Such phenomena indicate that the structures of the hemoglobin molecules are quite different; in fact, more differentiated than the molecules of members of an isomorphous group of simple carbonates, such as the carbonates of calcium and magnesium which when in separate solutions crystallize in rhombohedrons whose corresponding angles differ 2° 15′, but which when in molecular union, as in the mineral dolomite, crystallize as a single substance which has an intermediate angle.

Upon the basis of our data it is not going too far to assume that it has been satisfactorily demonstrated theoretically, inferentially and experimentally that at least this one substance (hemoglobin) may exist in an inconceivable number of stereoisomeric forms, each form being peculiar to at least genus and species and so decidedly differentiated as to render the hemoglobin crystal test more sensitive in the recognition of animals and animal relationships than the zooprecipitin test.

Subsequent to the research referred to, investigations have been pursued in the study of hemoglobins from various additional sources, especially from representatives of *Primates,* with the result in the latter case of finding indubitable evidence of an ancestral alliance of man and the manlike apes.

Such studies as those of Reichert and Brown are valuable as affording a new and exceedingly delicate means of establishing differences and relationships between various hemoglobin-possessing forms, and somewhat similar methods serve to differentiate between starches.[25] The results so far obtained have largely served to establish the fact that the classifications that have been set up in the plant and animal kingdoms on the basis of morphology are well justified by these chemical studies. It is probable that these methods may serve to settle disputed points of relationship, as in the case of the albino rat, and perhaps will lead to some readjustment of existing classifications. But on the whole one must admire the accuracy with which the morphologists have done their systematization, and marvel at the fact that morphologic differentiation corresponds with, and presumably depends on, chemical differences. Of course there are found discrepancies between the structural and the chemical relationships. As Newman[26] said:

For that matter there is no parallelism between classifications based on teeth and that based on feet, or between any two other unconnected systems. No two systems of an organism exactly keep pace in evolution. One may remain relatively conservative, while another undergoes pronounced specialization. Of all systems the blood seems to be the most conservative and to have retained longest its ancestral condition. It is on this account that blood tests so often reveal relationships that can scarcely be revealed in any other way.

25. Carnegie Institution of Washington, D. C., publication 173; rev., Am. J. Botany **3**:91, 1916.

26. Newman, H. H.: The Gist of Evolution, New York, The Macmillan Company, 1926.

IMMUNOLOGIC EVIDENCE

Of even wider applicability, since they require no crystallization or other physical constants, are the methods of immunology, which itself constitutes one of the most important of the branches of biochemistry.[27] The serologic reactions are essentially delicate methods for differentiation of proteins (either alone or as the complexes existing in protoplasm), and since the evolution of different species is essentially the evolution of different combinations of amino-acids to form the proteins characteristic of each species, and the carbohydrate and fatty compounds that help them to make up cells, evidently the serologic reactions afford a means for the analysis of the evolutionary relationship of species.

This opportunity was recognized in the earliest days of immunology by that pioneer in the study of the precipitin reaction, George H. F. Nuttall of Cambridge University. The precipitins were first studied in 1897 by Kraus, and in 1904 Nuttall [28] published his monograph in which he recorded 16,000 precipitin tests made with the blood of a vast range of animal species. This pioneer work of Nuttall's blazed a broad trail which, for some unknown reason, has been little followed. Since that time methods have been improved, so that much more clear distinctions and more quantitative results are obtainable, yet few applications of these methods have been made to problems of zoological and botanic differential classifications.

In general, the results obtained by Nuttall were in accordance with the statement made previously, that the existing morphologic classification corresponds well with the chemical differentiation. For example, antiserum for the blood of domestic cats gave the strongest reaction with the blood of cats, weak reactions with other carnivora, and none at all with the blood of primates, ungulates and marsupials. Antidog serum gave a stronger reaction with the blood of jackals than with blood from less closely related carnivora. Antipig serum gave no significant reactions with the blood of many other mammals, including whale blood, despite the tradition of some relationship between these two dissimilar mammals. Antillama serum showed the relationship of this animal to the camels. Antimarsupial serum established the relationship of several members of this group, and the lack of relationship to other mammals. Antiserum for the blood of fowls and ostriches showed a general relationship of the serum of many kinds of birds, a slight relation to reptiles and almost complete distinction from the mam-

27. Wells, H. Gideon: Immunology as a Branch of Chemistry, New York, Columbia University Press, 1927.

28. Nuttall, G. H. F.: Blood Immunity and Blood Relationships, London, Cambridge University Press, 1904.

mals. So too, antiserums for blood and egg proteins showed strong relationships between turtles, alligators and crocodiles, and a slight but distinct relationship of these reptiles with the birds, corroborating the paleontologic evidence of the origin of the birds from the reptiles. Antiserum for lobster blood reacted only with the blood from decapods. An interesting result was obtained by Graham-Smith with antiserums for *Crustacea* and for the king-crab, *Limulus*, for it was found that the limulus, famous as a "living fossil" because it has come down almost unchanged for so long a geologic history, is not related to the crab at all, but to the spiders, a conclusion that had also been advanced on anatomic grounds.

This work was done by relatively crude methods that did not permit the establishment of the minor degrees of relationship and distinction that are possible by methods now available, and there is no doubt that much more valuable evidence is to be obtained by any one who again ventures into the field with refined methods. For example, several investigators have found that immunologic comparisons of the blood of rats and that of mice shows them to be much less closely related than their general similarity of appearance and habits would indicate.[29] And Kritchevsky [30] has adduced by the method of complement fixation some evidence that the embryonic frog in the tailed tadpole state is intermediate between the frog and the tailed amphibian, *Triton*, which may be looked on as a precursor of tailless amphibians.

The limited studies of quantitative relations with the precipitin reaction by Boyden,[31] as far as they went, in general confirm Nuttall's observations, and Boyden's conclusion is that "the degree of reaction between an antiserum and various animal bloods has in general paralleled the relationship between those animals. Thus nearly always the reaction of a given antiserum is greater with animals belonging to the same order than with members of other orders." He emphasized the desirability of applying immunologic methods to the zoologist's problems in respect to the relationship of the animals now in dispute.

It must be admitted that the results of phylogenic studies as yet obtained by means of simple immunologic tests do not contradict the conclusion of Erhardt [32] that, although useful for such studies, serologic methods are less valuable than the anatomic comparisons. But the possibilities of such investigations have been enhanced by recent investigations on specificity, especially those of Landsteiner, which have

29. Steffenhagen and Schoenburg: Ztschr. f. Immunitätsforsch. u. exper. Therap. **8**:563, 1911.

30. Kritchevsky: Centralbl. f. Bakteriol. **72**:81, 1914.

31. Boyden: Biol. Bull. **50**:73, 1926.

32. Erhardt: Ztschr. f. Immunitätsforsch. u. exper. Therap. **60**:156, 1929.

brought out the fact that animals possess two sorts of specific substances, simple proteins and complex antigens, the latter of which are proteins combined with other substances, especially lipoids. Landsteiner [33] said:

> Aside from the chemical study of antigens it was noticed that the specificity of the serologic reaction of blood cells obeys other rules than does the species specificity of proteins as shown by precipitin reactions. This led to the conclusion that there exists a two-fold system of species specificity in animals. In the case of the proteins, the serological and chemical properties in general parallel closely the zoological relationship and the changes are gradual (Nuttall, Reichert, and Brown). Thus one could roughly construct the zoological tree merely on the basis of precipitin reactions. The specificity of cells is characterized on the contrary by the occurrence of so-called heterogenetic reactions which disclose the existence of similar structures in unrelated species and at a glance would indicate a mosaic structure.
>
> A second distinguishing feature is the fact that allied species as a rule can be differentiated by hemagglutinins but not by precipitins. This is hardly caused by the unequal sensitivity of the two methods only, because with erythrocytes the said differences are considerable in comparison with those exhibited by distant species.
>
> That individual protein differences occur has been assumed also on the basis of purely chemical investigations. Meyer and Treudtel found differences in the flocculability and the isoelectric point of casein prepared from the milk of various women. The existence of more than one form of hemoglobin in the blood of one species was reported by Küster and by Anson, Barcroft, Mirsky and Ouinuma. According to the claims of Falkenhausen and Fuchs, racial differences in proteins can be detected by the action of digestive serum ferments. It will be of importance to repeat and extend these studies and to ascertain whether the findings bespeak constitutional and hereditary variations of proteins among individuals, analogous to those which have been found to exist in the complex cell antigens or their haptens. If the answer is in the affirmative one should rather expect to obtain parallel results by serological reactions.
>
> Since species are characterized not only by morphological attributes but by their specific biochemical constitution as well, it seems evident that the somatic and functional development of the organic world was paralleled by a biochemical evolution of the proteins and haptens. One must assume that the two lines of events are linked in some way although no explanation has been offered as to how such a correlation might be brought about. In this regard it is essential to know whether the small initial steps in evolution are coupled with changes in the proteins. If so, individual and racial protein differences, perhaps too small to be detected by the methods available, ought to be a matter of regular occurrence. In the opposite case it is conceivable that the transformation of proteins came about discontinuously, contingent upon the occurrence of numerous changes or modifications of a special kind in the germinal constitution, perhaps in connection with hapten variations. It is too hazardous, attractive as it should be, to speculate further along these lines. Yet to perceive the problem may not be superfluous and may lead to experimental investigation.

33. Landsteiner: J. Immunol. **15**:589, 1928.

So far the chief reinvestigation of Nuttall's pioneering contribution has been of the relationship of the primates, because of the burning interest in this subject. A considerable part of Nuttall's work was with primate blood.[34] Testing antihuman serums (which were so strong that they were not very specific, giving slight reactions in 14 of 97 carnivora and 11 of 40 ungulata), he obtained results in testing with the blood of 97 different primates as shown in table 2.

From this it is seen that the blood of the chimpanzee and that of the orang-utan reacts fully as well with antihuman serum as does human blood itself, in contrast with the bloods of lower primates,[35] which gave much less marked reactions. With a weaker and therefore more specific antihuman serum, no reactions were obtained with the blood of the lower monkeys. An antichimpanzee serum gave strong reactions with the blood of man, chimpanzee and orang-utan, but not with that of the lower primates.

TABLE 2.—*Results of Testing Blood of Primates with Antihuman Serums*

Samples	Species	Degree of Reaction		
		Slight	Medium	Strong
34	Human (4 races)	3	7	24
8	Higher apes—Simiidae (3 species)	0	0	8
36	Old World monkeys—Cercopithecidae (26 species)	26	3	4
13	New World monkeys—Cebidae (9 species)	5	3	0
4	Marmosets—Hapalidae (3 species)	1	0	0
2	Lemurs—Lemuridae (2 species)	0	0	0

34. A few previous observations on crossed immunologic reactions between human blood and blood of lower primates had been reported by Wassermann, Grünbaum, Biondi and Ewing. Grünbaum alone compared human, chimpanzee, orang-utan and gorilla and reported that it seemed impossible to differentiate between the bloods from these four species. Friedenthal in 1905 also found it impossible to distinguish between the same four species by the precipitin reaction. In 1907, Bruck reported that according to complement fixation tests antiserum for orang-utan blood showed about the same relation to human blood as to Macacus blood, but was closer to gibbon (Hylobates) blood. Furthermore, he reported finding quantitative differences between the bloods of Europeans, Malays, Arabians and Chinese (Berl. klin. Wchnschr. **44**:793, 1907; Arb. a. d. k. Gsndhtsamte **37**:618, 1907).

35. The quantitative comparisons made with the bloods of primates and their antiserums by Nuttall and Strangeways are not of value because of unsatisfactory methods. Hektoen (The Precipitin Test for Blood, J. A. M. A. **70**:1273 [May 4] 1918) reported a series of precipitin tests with an antihuman serum which gave a reaction with human blood in a dilution of 1:5,000, and with the blood of Macacus rhesus in a dilution of 1:100, whereas a large series of other mammalian bloods reacted only at 1:10. Also, he found that Rhesus hemoglobin alone of numerous mammalian hemoglobins examined (not including hemoglobins of primates higher than Rhesus) gave strong precipitin reactions with antiserum for human hemoglobin (Hektoen and Schulhof: J. Infect. Dis. **31**:32, 1922; **33**:224, 1923).

In conclusion, Nuttall said: "if we accept the degree of blood reactions as an index of the degree of blood relationship within the anthropoidae, then we find that the old world apes are more closely allied to man than are the new world apes, and this is exactly in accordance with the opinion expressed by Darwin."

This is also in agreement with Reichert's observations on the hemoglobins, concerning which, with his permission, I quote from a letter recently received from him:

> You will note in the Hemoglobin Memoir that very little attention was given to the hemoglobins of the human being and other members of the Primates. This scanty reference was due to the fact that I was preparing an elaborate memoir on the hemoglobins of the Primates, in which I had planned to go very fully into the study of the human hemoglobins, making especially exhaustive studies of the 2 *different forms* of hemoglobin *normally* present in the *human blood.* Such duality was found in certain other bloods, very striking and beautifully exhibited in the blood of the *horse.* I had done sufficient work to convince me that the hemoglobins of each genus examined were readily distinguishable from those of other genera, and that there were very positive lines of demarcation between the hemoglobins of lemurs, monkeys, baboons, apes, etc., and, moreover, that the hemoglobins of the anthropoid apes were strikingly closer to those of man than in the case of the hemoglobins of the other groups. I never published any results because I was unable for various reasons to bring my investigations to a satisfactory stage of completion.

The chief recent work in this field is reported in three papers by Landsteiner and Miller.[36] Having observed that antierythrocyte reactions with immune serums serve to differentiate readily between species so closely related that their blood serums are not distinguishable by the precipitin reaction, they investigated the bloods of primates by means of immune antiserums, which agglutinate erythrocytes. By this means it was possible to show that while there is a close relation between the bloods of man, chimpanzee and orang-utan (gorilla blood was not tested), nevertheless by proper technic (saturation method) it was possible to distinguish between them, although this method did not serve to distinguish between the blood of white persons and that of negroes. These experiments also indicated that the difference between the blood of man and that of the chimpanzee or the orang-utan is less than between either of them and the lower monkeys. Furthermore, the blood of the anthropoids is not much if any more similar to that of the lower monkeys than is the blood of man to that of the lower monkeys. They stated that "this conclusion is in agreement with the accepted view of zoologists that man has not evolved directly from any of the existing primates, as was formerly supposed, but that the Catarrhina (Old World mon-

36. Landsteiner, K., and Miller, C. P., Jr.: Serological Studies on the Blood of the Primates, J. Exper. Med. **42**:841, 853 and 863, 1925.

keys), anthropoids and man have all sprung from a common stock." They supported the view that the present anthropoid apes, closely related to man as they are, do not represent direct intermediates between monkeys and man.

How much alike the bloods of man and ape really are, is strikingly shown by the observation that there is as much or more difference between the blood of horse and that of donkey in respect to immunologic behavior, as between the blood of man and that of chimpanzee, and horse and donkey are so closely related that they can breed with one another, even though the product of their mating is but the lowly, infertile mule, "without pride of ancestry or hope of posterity."

Landsteiner and Miller also studied anthropoid blood in respect to the occurrence of blood groups, which have been shown to vary among the different human races, as indicated by their agglutinating effects on one another. It was found that the bloods of anthropoid apes contain groups identical with those of human blood. Of seventeen chimpanzees, three had blood of group 1; fourteen, of group 2; three, of group 3, and one, of group 4. On the other hand, the occurrence of red cell agglutinogens of the human type seems to be limited to man and the anthropoids, being differentiated from the agglutinating factors present in the blood cells of lower monkeys and other mammals. Landsteiner and Miller discussed the significance of their observations as follows:

> The presence of the blood group factors in the apes has evidently a bearing on the question of the significance and origin of the blood groups in man. The literature on the subject contains two chief hypotheses, one that the blood groups were present in the different races of primitive man, the other that they arose as relatively recent mutations. If our findings in the anthropoids are taken into consideration, the simplest assumption seems to be that the isoagglutinable factors existed before man and the anthropoids were differentiated from their common ancestor. If this assumption is not made, one is forced to the conclusion that identical mutations occurred in the evolutionary lines which developed into the gibbon, orang, chimpanzee, and man at some later time.
>
> Paleontological studies have led to the generally accepted conclusion that the existing species of man is only one of a number of human species, the rest of which have become extinct, as for instance the Neanderthal man. If the group factors had evolved before the human stage had been reached they must have been present in these various fossil human species. Either the groups were distributed among them in a manner comparable with the distribution in man of the present day or else in such wise that each species contained only a part of them.
>
> In contemplating the latter case the question presents itself whether or not the crossing of human types more or less pure as regards the groups may have resulted in the puzzling existence in man of a few sharply defined serological qualities and in their distribution. In this respect the findings in anthropoid apes are of interest. In chimpanzees only the isoagglutinogen A (of Group II cells) was encountered. Although the number of individuals examined is not large, and more extensive studies are needed, it is probable that the factor A is either characteristic for these animals or is predominant, and is present in a higher proportion

than in any known race of man. A parallel case, which can be cited, is that of the North American Indians whose bloods are almost entirely of Groups I and II.

According to Semzerva and Terechowa,[37] the human fetus does not possess these specific group antigens until after the sixth month of gestation, an observation which fits well with the evidence of "comparative immunology" that these characteristic human and anthropoid antigens are absent from the lower monkeys—another illustration of the recapitulation of phylogeny by ontogeny. That the biochemical structure that makes up the immunologic pattern develops during structural development is also indicated by the observation of Kritchewsky[38] that heterogeneous antigen for sheep erythrocytes (Forssman antigen), which is abundant in the blood and tissues of adult fowls, does not appear in the developing egg until after the fourth day of incubation.

It is unfortunate that one cannot study the immunologic relationships of fossil forms that are supposed to be ancestors of existing creatures. The only instance in which this has been possible has been reported by Friedenthal,[39] who found that the blood of a Siberian mammoth which had been preserved in the natural cold storage plant of arctic ice for perhaps 25,000 years gave immunologic reactions establishing its close relationship with modern elephants. Immunologic tests with extracts of the dried blood and tissues of Egyptian mummies have shown that such extracts give immunologic reactions with antiserum for modern human blood and sensitize animals to human proteins.[40] This indicates merely that the properties that determine specificity can resist desiccation for from 2,000 to 5,000 years, and that no marked change in human proteins has taken place in that relatively short period of time.

It would be extremely interesting, if it could possibly be done, to study the immunologic reactions of extracts from insects found in amber, for Wheeler has found that ants preserved in amber believed to be millions of years old belong to genera of ants existing at the present time. So much did those ancient ants resemble the moderns that with them in the amber have been found the plant lice that they tended just as certain ants do now, and even similar parasites. Such persistence of forms without change supports Moodie's[41] conclusion that a disease found in the jawbone of a fossil rhinoceros was essentially the same as the actinomycotic lumpy jaw of present-day cattle.

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37. Semzerva and Terechowa: Klin. Wchnschr. **8**:206, 1929.
38. Kritchewsky: J. Infect. Dis. **32**:192, 1923.
39. Friedenthal: Deutsche med. Wchnschr. **30**:901, 1904.
40. Uhlenhuth: Ztschr. f. Immunitätsforsch. u. exper. Therap. **4**:761, 1910.
41. Moodie, R. L.: Paleopathology, Urbana, Ill., University of Illinois Press, 1923, p. 250.

It is probable that other and better opportunities may be found for studying evolutionary problems by immunologic methods if they are sought for. For example, immunologic study of forms of life that have persisted practically unchanged for vast periods, such as the horseshoe crab, the pearly nautilus and some of the mollusks, and of the related forms that have apparently been derived from these 'living fossils" or their relatives, might give extremely interesting information.

EVIDENCE FROM IMMUNOLOGIC RELATIONSHIPS OF VEGETABLE PROTEINS

The vegetable kingdom may well offer possibilities that have so far been untouched. General observations on the immunologic behavior of plant proteins and crude extracts of plant tissues have shown that they behave like animal materials in respect to specificity, i. e., the strongest reactions are obtained with the same materials as are used in immunizing or sensitizing, weaker reactions are obtained with materials from closely related species and no reactions with materials from dissimilar species. This depends on the frequent presence of chemically similar antigens in closely related species,[42] and their infrequency in unrelated species. The most extensive study by immunologic methods of the relationship of plants has been made by Mez and Ziegenspeck,[43] who have worked out in this way a scheme of botanical classification, which, as with animal material, shows that morphologic and biochemical (immunologic) classifications agree closely and therefore, presumably, rest on a common basis. This basis presumably is that variations in anatomic structure are associated with or dependent on differences in chemical composition.

Lewis and I [44] have had the opportunity to cooperate with Professor Gortner of the University of Minnesota in a study of the immunologic relations of certain alcohol-soluble proteins from cereal grains, with results that suggest further possibilities in respect to the use of immunologic methods in the study of evolutionary history.

Proteins soluble in relatively strong alcohol (from 70 to 80 per cent), called prolamines because rich in prolines, are not common, being found chiefly in the cereal grains, gliadin of wheat and zein of corn being perhaps the best known. A number of others have been isolated and studied by Gortner and Hoffman. They were obtained from wheat, durum, emmer, einkorn, oats, barley, spelt, corn, kafir, teosinte and sorghum seeds. The chemical properties, as known from such data as

42. Wells and Osborne: J. Immunol. **19**:183, 1916.

43. Mez and Ziegenspeck: Botan. Arch. **13**:483, 1926; résumé by Mez, Leopoldina **2**:132, 1926.

44. Lewis, Julian, and Wells, H. Gideon: J. Biol. Chem. **66**:37, 1925.

are contained in the literature and those obtained by Gortner and Hoffman, indicate that these proteins may be divided into a "wheat group," which includes the proteins isolated from the genus *Triticum,* and a "corn group," including those isolated from maize, teosinte, kafir and sorghum.

The genetic behavior and nuclear structure of these cereals have been extensively studied by plant breeders, especially with the wheat group, and these studies seem to reveal interesting evolutionary relationships. It has been shown that einkorn, apparently the ancestor of modern wheat, is characterized by having seven chromosomes, that emmer and durum, presumably intermediate stages, have fourteen chromosomes, and that wheat and spelt have twenty-one chromosomes, as if their evolution had been accomplished by polymerization.[45] The fertility or total or partial sterility of interspecies is said to be in accordance with these chromosome numbers. In the "corn group," it has been found that teosinte will hybridize with ordinary corn, indicating a genetic relationship.

Our immunologic results agree perfectly with the conclusions drawn from the chemical data of Gortner and Hoffman, who found that the chemical properties of these alcohol-soluble proteins indicate the existence of a "wheat group" and a "corn group of prolamines, the members of each group being chemically similar to each other. Our results also showed that these alcohol-soluble proteins from cereal grains fall into these two classes. Gliadin from wheat and rye and the prolamines from durum, einkorn, emmer and spelt, of the genus *Triticum,* seem to be closely related, apparently identical, according to all our tests. The alcohol-soluble proteins from common maize, from kafir corn and from teosinte were found to be closely related to one another, but not to the alcohol-soluble proteins of the group *Triticum.*

Accepting the morphologic evidence that modern wheat with its twenty-one chromosomes has developed from the simple einkorn with seven chromosomes, perhaps through a stage in which there were fourteen chromosomes, as illustrated by durum and emmer, one finds that through these evolutionary stages one of the most abundant and characteristic proteins of the seeds has retained a constant chemical composition.

EFFECTS OF CHEMICALS IN ALTERING DEVELOPMENT

The knowledge of immunologic processes, as well as the application of immunologic methods, also have made some, and probably will in

45. Such mutation by polymerization has been observed in other species of plants. Thus de Vries observed that a primrose with fourteen chromosomes produced two robust mutants with twenty-one and twenty-eight chromosomes, respectively.

the future make more, contributions to the study of the processes of evolution. One thing they have done is to provide strong evidence that the strict Weismannian conception of the eternal continuity, sanctity and inviolability of the germ plasm is untenable, and that more is to be said for at least a modified form of Lamarck's doctrine of inheritance of acquired characters than a previous generation of biologists was willing to admit. Adami,[46] in his wartime Croonian lectures, delivered in 1917, brought forward various illustrations familiar to medical men, of the transformation of an acquired cellular character into a permanent one, transmissible to subsequent generations of cells. One of these is the striking and familiar example of the behavior of cells transformed in character, i. e., metaplasia. As every sophomore medical student is supposed to know, under the influence of chronic repeated injury, secreting columnar epithelium may resign its secretory character to take up a protective structure and function in the form of stratified, hornified epithelium. If the continued injury leads to the development of a cancer in this tissue, this cancer consists of stratified epithelium with abundant keratin, exactly like a cancer derived from the stratified epithelium of the skin or mouth. Here a recently acquired change of character and chemical composition of adult cells is perpetuated in their offspring which, before the death of their victim, produce innumerable generations of descendants all carrying this artificially impressed character.

Adami also pointed out the persistence of transmission of acquired characters in bacteria. For example, it takes days and sometimes weeks before bacteria take on the active dissociation of a foreign sugar, glucoside, alcohol or fat, but once this power is acquired it is apt equally to be retained for generations in the absence of that particular fermentescible substance.

The immunity to infectious diseases seems to represent a similar ability of cells to transmit an acquired character to an indefinite number of subsequent generations of somatic cells. For example, a child acquires immunity to smallpox, measles or whooping cough, and seventy years later the tissues of that individual, the descendants of the cells that acquired whatever property it is that confers this resistance, still exhibit the same immunologic capacity. Here is an acquired property that is transmitted indefinitely in the multiplying somatic cells.

Now if the tissue cells can have reproducible qualities impressed on them by environmental conditions, how can the germ cells in the same body, nourished by the same blood carrying the same influences, escape being influenced? The germ cells have to be formed from the materials

46. Adami, J. G.: Medical Contributions to the Study of Evolution, London, Duckworth & Company, 1918.

brought by the blood, and at least in the case of the male germ cells new formation is going on throughout life. It has been estimated that in the course of a reasonably active sexual life a man produces about from 300 to 500 billion spermatozoa, each one of which carries just as much power to determine the characteristics of the offspring as any one of the 400 or so ova that mature in the life time of a woman. And there is plenty of evidence that the spermatozoa, as well as the ova, may be so altered that acquired properties become inheritable. For example, there are the classic experiments of Stockard [47] showing that the scanty, runty offspring of alcoholized guinea-pigs when bred together produce in subsequent generations offspring with defective eyes, an observation in keeping with the generally recognized tendency for the existence of defects in the descendants of alcoholic human progenitors. And corroborating these are the observations of Arlitt [48] that the descendants of heavily alcoholized rats produce offspring of low mentality. Undoubtedly, these results depend on the fact that alcohol is a poison which, like lead, attacks strongly the testicles, for notoriously the testicles of human alcoholics show profound atrophy and loss of spermatogenesis, and in Arlitt's rats the testicles showed atrophy in direct proportion to the degree to which the rats had taken alcohol.[49] Furthermore, there is the evidence that injury to the germ cells by x-rays and radium leads to transmissible defects in the progeny, Bagg and Little [50] having bred mice thus affected with defects of the eyes and the feet through ten generations and obtained families with as high as 100 per cent defectives. The abnormalities were truly inherited, behaving as mendelian recessive characters.

In fruit flies (*Drosophila*), studies of the effects of x-rays have been particularly instructive, because in these creatures it is possible to observe the changes produced in the chromosomes which lead to the production of abnormal progeny.[51] Mavor's [52] experiments show that of all parts of the cell the hereditary material responds most easily to the effects of x-ray treatment, for exceptional flies that develop from eggs treated with x-rays are normal except in respect to their hereditary behavior. They prove that an external agent can "modify the mechanism of inheritance in such a way that a permanent effect is produced which

47. Stockard: Am. Naturalist **47**:641, 1913; **50**:65 and 144, 1916; J. Exper. Zool. **26**:119, 1918; Tr. Am. Philosoph. Soc. **62**:311, 1923.

48. Arlitt, Ada H.: The Effect of Alcohol on the Intelligent Behavior of the White Rat and Its Progeny, Psychol. Monog. 1919, vol. 26, no. 4,

49. Arlitt and Wells: J. Exper. Med. **26**:769, 1919.

50. Bagg and Little: Am. J. Roentgenol. **10**:975, 1923; Am. J. Anat. **33**:119, 1924.

51. Muller: Science **66**:84, 1927.

52. Mavor: Am. J. Roentgenol. **10**:968, 1923.

is transmitted through successive generations" and which depends on the production in the treated eggs of an irregular distribution of the chromosomes because of changes either in the chromosomes themselves or the protoplasm surrounding them. These changes in hereditary qualities may occur without alterations in the vitality of the cells and their progeny. The fact that it has already been found that x-rays can produce mutation of genes and reorganization of chromosomes not only in fruit flies, but also in wasps, barley and tobacco raises the question whether all the mutations that have led to the vast variety of living things may not have been due to rays of short wave length and high speed particles of corresponding energy content. Muller [53] suggested: "if so, biological evolution has been made possible only by the stray radiation present in nature—the beta and the gamma rays and the cosmic rays." The developments so far made in this intriguing field open up vast possibilities to the imagination.

IMMUNOLOGIC MODIFICATION OF DEVELOPMENT

Highly suggestive studies have been made on the possibility of another method for modifying hereditarily transmitted acquired characters, by Guyer and Smith,[54] who have used the technic of immunology in their investigations. They prepared antibodies for rabbit crystalline lens substance by immunizing fowls with pulped rabbit lenses. The antiserum was injected into rabbits at an early stage of gestation, at a time when the antibodies might possibly influence the developing lens elements of the fetal eyes. Although the mothers that received the injections suffered from no visible effects on their ocular tissues, it was found that occasionally some of their offspring had defective eyes. What is of more significance, if these offspring were bred with one another, larger and larger proportions of rabbits with defective eyes were produced in subsequent litters. The lesions consisted of opacities of the lenses, coloboma, microphthalmia, detachment of the retina, abnormal intra-ocular pressure and various abnormalities in the shape of the lenses and of the eyeballs.

Genetically, the defects of the eye behaved after the manner of a mendelian recessive character, although no pure defective-eyed strains that did not produce some apparently normal offspring had been produced in the first nine generations. However, with the later generations of inbred defectives, higher and higher proportions of offspring with abnormal eyes were being obtained. Controls with animals receiving other sorts of antibodies indicate that this phenomenon truly depends on the injury to elements of the developing lens by specific antibodies for lens substance.

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53. Muller: Proc. Nat. Acad. Sc. **14**:714, 1928.

54. Guyer and Smith: J. Exper. Zool. **31**:171, 1920; **38**:449, 1924.

Of particular significance for the whole vital problem of how inheritable variations arise from which, by natural selection or otherwise, new forms of life appear on the earth are later observations by Guyer and Smith. They took advantage of the fact, repeatedly demonstrated by immunologists,[55] that the antigenic proteins of the lens are in large part the same in different species, and not so specific for each species as are the blood proteins, so that animals can develop antibodies even for their own lenses. Rabbit lens substance was set free in the bodies of rabbits, and it was found that antibodies were produced, giving precipitin reactions with the lens substance from rabbit eyes. When such immunized rabbits reproduced, inheritable eye defects sometimes appeared in the offspring. Even needling the lens of the eye of a female rabbit may cause specific antibodies to appear in its own blood, and such rabbits sometimes produce young with defects of the eyes. Apparently to some extent, at least in rabbits, the placenta is permeable to specific antibodies which can attack not only the corresponding specific structures in the fetus, but also those elements of its germ plasm that are responsible for the formation of the same structures, for how otherwise could this new property be transmissible through succeeding generations like any inheritable recessive character? That it does not depend on injury to the original maternal germ plasm is shown by the fact that a rabbit which has had an injection with antiserum for lens and has born a litter with defective eyes will subsequently bear only normal offspring. That the change in the subsequent generation is due to permanent alterations in the germ plasm is proved by the fact that it can be transmitted by males, as well as by females.

Guyer cited one case in which a male and a female, *in utero* at the time the antilens serum was injected and born with apparently normal eyes, when bred together produced offspring with defective eyes, suggesting that their germ cells had been affected by the antibodies independent of any evident damage to their own eyes.

Although awaiting confirmation and amplification by other investigators, these observations are highly suggestive as to possible ways in which variations of previously existing forms of life may have been brought about. For example, may not the production of defects of the eyes in offspring of rabbits with degenerated crystalline lens point the way to the explanation of the old problem of why fish living in dark caves are blind, a fact which has often been referred to as an apparent illustration of the production of an hereditary character from an acquired character.[56] In these fish, the lens and other structures

55. Hektoen, L.: The Specific Precipitin Reaction of the Lens, J. A. M. A. **77**:32 (July 2) 1921; J. Infect. Dis. **31**:72, 1922; **34**:433, 1924.

56. Eigenmann: The History of the Eye of the Blind Fish Amblyopsis from Its Appearance to Its Disintegration in Old Age, Mark Anniversary Volume, New York, Henry Holt & Company, 1903.

of the eye are commonly more developed in the embryos and young individuals and degenerate with age, possibly leading to the formation of antibodies with injury to the germ plasm, as in Guyer's rabbits with disorganized lenses. May not injury to other tissues than lenses also lead to the formation of antibodies capable of producing alterations in the germ plasma?

Such observations and considerations support strongly the views expressed by Adami and others [57] that the germ cells can be affected by environment, especially by chemical agents, such as toxins, antibodies, hormones, alcohol, lead and such physical agents as the emanations of radium and roentgen rays, and that in some cases, at least, the alterations thus produced when not incompatible with reproduction may persist as fixed properties in the genes. Since apparently most of the effects thus produced are detrimental and reduce the chance of survival, they tend ordinarily to self-elimination rather than perpetuation, but many congenital deformities and abnormalities seen in man may well have had such an origin, just as clinical observation has repeatedly suggested that parental alcoholism may lead to physical and mental abnormalities in the progeny.

On the other hand, certain beneficial modifications might be produced in the same way, which would tend to be preserved through natural selection. Possibly it is in some such way that species acquire a heightened capacity to resist disease, for it has been abundantly and tragically demonstrated that races or groups exposed for the first time to an infectious disease show much less capacity to resist this disease than populations long exposed to this infection. Preliminary experiments by Guyer and Smith [58] indicate that the offspring of animals immunized to typhoid bacilli for three or four generations had greater capacity to produce antibodies for typhoid bacilli than did the animals of the first generation.

When one considers the excellent work that has been done, especially in Morgan's laboratory, in tracing the changes in the chromosomes that are responsible for the production of new, inheritable characters, one must also appreciate that, after all, the chromatin is a chemical compound, and whatever it does it accomplishes by virtue of the chemical properties of the chromosomes. Therefore there always is the possibility that new light may be thrown on heredity and evolution by chemical studies, whether by use of immunologic, physiochemical, analytic or synthetic methods. Schmalfuss [59] has presented an interesting dis-

57. Cunningham, J. T.: Hormones and Heredity, New York, The Macmillan Company, 1921.

58. Guyer and Smith: J. Infect. Dis. **33**:498, 1923.

59. Schmalfuss: Naturwissenschaften **16**:209, 1928.

cussion of the theoretical possibility of considering heredity from a chemical standpoint. It has been suggested that the agents responsible for heredity may be autocatalysts, which hasten or delay the reactions in which they participate. The chromomeres behave much like catalyzers in high local concentration through adsorption on a colloid matrix. Whatever they are, they seem to possess a remarkable constancy of behavior, and therefore of composition. Nevertheless, like all the elements of the living cells they are subjected to the influences of their environment, which, as has been shown, evidently do at times modify them. If the alterations are of reversible character, as chemical changes commonly are, then the original condition may be restored as a mutation of the new forms. As chemical examples of such an alteration, Schmalfuss mentioned polymerization and depolymerization. I have already pointed out the apparent polymerization of the chromosomes in the wheat family and the primroses, and E. Baur has assumed the same thing in the color modifications of snapdragon (*Antirrhinum L*). Another chemical analogy is furnished by the mutual buffering and debuffering of salt mixtures. Of course, physical alterations may also be reversible, as in the change from gel to sol and the reverse. As Schmalfuss pointed out, the recognized linear arrangement of the hereditary substance in the chromosomes is not incompatible with the occurrence of physical alterations without change in the order of the different factors, just as in a string of pearls individual pearls may be rotated or the entire string altered in shape by bending, without altering the linear arrangement. He also pointed out how in experimental production of melanin by the action of enzymes, oxygen and water on dioxyphenylalanine the various types of melanin formation or its absence (albinism) that are familiar in studies of genetics can be simulated on a purely chemical model, with hybrids, dominants and recessives. He predicted that in the near future a systematic consideration of "developmental chemistry" will arise, throwing light on the problems of heredity and evolution.

Even now one sees from these first feeble steps in the lives of new sciences that their application to the problem of the origin of life and its evolution warrants hope that from this unexpected direction may come also weighty contributions to knowledge as to how some pathologic states may arise, and also how living forms have been able to evolve mechanisms protecting them from their environment and their diseases. The student of biologic evolution may give fully as much to medical science as biochemistry and immunology promise to give to him when he has learned to use these tools. Any progress in any one division of the biologic sciences carries along with it all the other divisions, and most surely the all-including division of medicine.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths.—Clarence C. Little has been elected president, Francis Carter Wood vice-president, and William H. Woglom secretary-treasurer of the American Association for Cancer Research.

Henry Albert, professor of pathology and bacteriology in the University of Iowa from 1903 to 1922, and commissioner of health for Iowa since 1926, died April 6, 1930, following an operation for appendicitis, at the age of 52 years.

Raymond C. Parker, formerly associated with cancer research at the University of Pennsylvania, has joined the scientific staff of the Rockefeller Institute for Medical Research.

C. C. Twort has been appointed director of cancer research at the University of Manchester.

Katsusaburo Yamagiwa, professor of pathology in Tokyo Imperial University, who produced tar cancer in rabbits—a wonderful achievement—died March 2, 1930. It is hoped to give more details about Professor Yamagiwa in a subsequent issue.

The following appointments are announced by the department of ophthalmology, Washington University, St. Louis, in connection with research on trachoma and other ocular infections: George H. Bishop, professor of applied physiology; Louis A. Julianelle, associate professor of applied bacteriology and immunology; James A. Hawkins, associate professor of applied biochemistry; R. W. Harrison, instructor in applied bacteriology and immunology; G. Bowers, assistant in applied bacteriology.

The Cameron Prize of the faculty of medicine of the University of Edinburgh for 1930 has been awarded George R. Minot and William P. Murphy, Boston, for their work on the treatment for pernicious anemia with liver.

Further Provisions for Research on Cancer.—On March 17, 1930, a new institute for cancer research was opened at Villejuif, near Paris. A new hospital is in course of construction beside the institute.

The legislature of Saskatchewan has established a permanent cancer commission to secure better education of the public in regard to cancer, and better facilities for the diagnosis and successful treatment of cancer.

Symposium on the Kidney.—A symposium on the kidney in health and disease is to be held in Minneapolis, July 7 to 18, 1930, under the auspices of the medical school of the University of Minnesota. The object is to secure better correlation of views and cooperation between the many highly specialized workers on problems connected with renal physiology and pathology.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

THE EFFECT OF SUCCESSIVE RADIATIONS WITH THE MERCURY ARC ON THE WHITE BLOOD COUNT OF NORMAL RABBITS. MARY HARDY and JEANNETTE CHAPMAN, Am. J. Hyg. **10**:655, 1929.

It was found that although single massive doses of ultraviolet radiation produced characteristic changes in the blood count, a dose of 12 lithopone units given daily for eight days produced no appreciable change in the total leukocyte or lymphocyte and polymorphonuclear counts.

JOHN PHAIR.

THE RELATIONSHIP OF THE CERVICAL SYMPATHETIC NERVES TO THE CEREBRAL BLOOD SUPPLY. STANLEY COBB, Am. J. M. Sc. **178**:528, 1929.

All the evidence taken into consideration, it would seem that vasomotor control of at least the superficial cerebral vessels is now established. One must be guarded, however, in drawing clinical conclusions. Physiologically, it has been shown that pial vessels may constrict or dilate in response to appropriate stimulation, but in anesthetized animals the changes are not of great magnitude, and may be overcome by large variations in systemic pressures. L. Hill's statement, strictly speaking, still holds: "There is no evidence of a causation of cerebral anemia by spasm of the cerebral arteries." Real, obliterating angiospasm has only been experimentally observed when vessels are strongly and locally stimulated under nonphysiologic conditions (Florey, 1925; Wolff, 1927; Lenox and Cobb, 1928). Recent investigations, however, certainly make vascular spasm a much more reasonable working hypothesis than it was thirty years ago.

AUTHOR'S SUMMARY.

RELATION OF THE ADRENAL GLAND TO THE TOXEMIA OF INTESTINAL OBSTRUCTION. R. A. CUTTING, Arch. Surg. **19**:272, 1929.

Bilateral adrenalectomy was performed on a series of rabbits, and material from obstructed loops of the intestine of other rabbits was injected into these and their controls. It was found that the adrenalectomized rabbits, although they did not exhibit as severe symptoms as their controls, nevertheless succumbed much more rapidly to toxemia. From his experiments, the author feels that the adrenal glands subserve a definite function in combating the toxemia of intestinal obstruction. Animals with intestinal obstruction showed early degenerative changes in the adrenal glands, so that if these glands do have a detoxifying activity, the attack on intestinal obstruction should be made early to avoid damage to the adrenals.

N. ENZER.

INTESTINAL OBSTRUCTION. F. RAINE and M. C. PERRY, Arch. Surg. **19**:478, 1929.

The authors worked on rabbits. They found that rabbits die of intestinal obstruction much more rapidly than dogs, largely because the former do not vomit. The release of the obstruction, when it had been made temporary, usually permitted the animal to return to health, and the reabsorption of the contents of the obstructed loop through normal intestine apparently initiated a rapid return to normal. This may be due to the reabsorption of the chlorides. When the pressure in the obstructed loop was reduced, the duration of survival was increased. They feel that release of pressure increases reabsorption and diminishes secretion. The therapeutic value of administering sodium chloride increases with the release of pressure and diminishes as the interintestinal pressure increases.

N. ENZER.

CORRELATIONS OF INTERNAL AND EXTERNAL PANCREATIC SECRETION. G. DE TAKÁTS and I. T. NATHANSON, Arch. Surg. **19**:771, 1929.

The article is subdivided into three sections, the first giving a review of the literature and an outline of the problem, and the second a description of the histologic changes which occur when the tail of the pancreas is isolated from the body by division either through ligature or more especially after the use of the cautery.

The observations may be briefly summarized as a progressive fibrosis and connective tissue replacement of the acini and ducts, with an apparent increase in the number of islands. The mechanism has its onset with an early edema and distention of the parenchyma, followed by a slowly progressive necrobiosis. The acini disappear first, and, while they do not entirely disappear, the ducts become small and widely separated. The connective tissue proliferation begins first between the lobules, and then becomes more diffuse and invading until finally the tail is represented by a dense, sclerotic cord. The islands are also involved in the edema, but as the sclerotic process progresses, new islet-like formations appear, and while not absolutely characteristic, these possess the staining properties and vascular arrangement of the island. In this experiment a portion of the tail, after separation, was transplanted into the omentum, and was found to survive for at least three months.

In the third division the effect on the blood diastase of ligating the tail was studied, and it was found that after ligation there was a short preliminary rise, followed in a few days by a larger, secondary rise in the diastase of the blood. The increase varies with the amount of the pancreas isolated, and if both the head and the tail are ligated, the rise is sudden and maximum and is not followed by a secondary increase. This test appears to be a sensitive index of acute pancreatic obstruction. In the dogs studied the diastase value was returned to normal usually by the end of two weeks.

N. ENZER.

OXYGEN CONTENT OF BLOOD IN PATIENTS WITH VARICOSE VEINS. A. BLALOCK, Arch. Surg. **19**:898, 1929.

The carbon dioxide and oxygen content of venous blood obtained from the femoral veins of normal patients and from varicose veins were compared. In unilateral varicose veins, it was found that the blood from the diseased vein had a higher oxygen content than from the normal vein on the opposite side. This was true of the blood obtained when the patient was in the standing or recumbent position. From normal veins, the oxygen content is highest when the patient is in the recumbent position, and decreases rapidly when he assumes the upright position. When the patient changed from the recumbent to the upright position, there was a smaller drop on the diseased side than on the normal side. It seemed also that the oxygen content was higher in the blood obtained from veins draining ulcerated areas, but too few cases were studied in respect to this. Similar results were obtained in a case of thrombosis of the inferior vena cava associated with dilated veins in the lower extremities. The author points out that these observations suggest an increase in the total flow of blood through a leg with varicose veins.

N. ENZER.

STUDIES ON THE EFFECTS OF OVERDOSAGE OF VITAMIN D. R. F. LIGHT, G. MILLER and C. N. FREY, J. Biol. Chem. **84**:487, 1929.

The administration of an excessive amount of viosterol may result in a drainage of mineral constituents from the body, with a relatively greater elimination of phosphorus than of calcium. The daily ingestion of 100,000 minimal effective doses may be followed in the rat, by anorexia, emaciation, greasy hair, labored breathing, and eventual death. No ill effects appear to attend the administration, to the rat, of 10,000 times the daily curative dose.

ARTHUR LOCKE.

THE PATHOLOGICAL ACTION OF LIGHT. A. G. LEVY, J. Path. & Bact. **32**:387, 1929.

The essential action of white light on sensitized tissues and of ultraviolet light on nonsensitized tissues appears to be identical. The result of prolonged irradiation of the mouse's ear is an immediate complete stasis, with subsequent necrosis. The result of less prolonged irradiation is a delayed stasis, with subsequent necrosis. In no case is stasis conditioned by the formation of clots. It is due to an inflammatory reaction, accompanied by extravasation of plasma from the vessels and consequent aggregation of corpuscles. The necrotic changes are a consequence of the cessation of circulation. The coagulation of extravasated plasma is a consequence of circulatory arrest. Hardening of the dead tissue is due to mechanical desiccation. Hypertrophy and infiltration of the epithelium are striking results of irradiation which is insufficient to produce general stasis. The hypertrophy is definitely shown to result from a brief period of irradiation.

AUTHOR'S SUMMARY.

EXPERIMENTS TO DETOXIFY CHLOROFORM. H. FÜHNER, Deutsche med. Wchnschr. **55**:1331, 1929.

White mice exposed for three hours to a certain concentration of chloroform vapor containing 1 per cent alcohol died on an average in one and a half days. Mice that were treated similarly but with 10 per cent alcohol added to the chloroform died usually in from four to six days. Alcohol inhibits the oxidation of chloroform to phosgene, and this is thought to account for the lower toxicity of chloroform to which more alcohol has been added.

PAUL J. BRESLICH.

GOITER IN RUSSIA: A GEOGRAPHICAL-PATHOLOGIC CONTRIBUTION. H. J. ARNDT, Verhandl. d. deutsch. path. Gesellsch. **24**:97, 1929.

One thousand one hundred and four specimens of normal thyroids and goiters were studied in different goiter-free and in most of the goiter districts of Russia. Endemic goiter was encountered not only in mountainous but also in level regions. In Moscow 18 per cent of the cases had adenomas, in Perm one fourth, in Tiflis two fifths and in Irkutsk about one half of the cases. The goiter-free thyroid gland in Russia does not show the small follicular structure, so typical for the thyroid gland in Switzerland. Only in White Russia and East Siberia, marked epithelial proliferation is noticed in the normal gland.

Most of the patients with goiter who were operated on in Moscow presented severe or mild thyrotoxic symptoms. The goiters obtained from these patients did not correspond with the classic histologic description of exophthalmic goiter, but were diffuse colloid goiters with epithelial proliferation. In Petrograd and Charkow, the conditions were much the same.

In the endemic region of White Russia, three fourths of the goiter material consisted of macrofollicular diffuse colloid goiters; in the Volga Valley, on the other hand, the nodular goiter was prevailing. In the Ural Mountains, the diffuse and nodular colloid goiter is common, without causing disturbance of the thyroid function. In the Northern Caucasus Mountains the diffuse colloid goiter with epithelial proliferation is common, and here it is often accompanied by thyrotoxic symptoms. In the territory of Tiflis (Transcaucasia) most of the goiters, obtained by operation, were adenomatous. In East Siberia, parenchymatous (fetal) adenomas prevail; clinically there are mild cases of hypothyroidism present. In Middle Asia (Teshkent) the adenomatous goiters are still more common and, in these regions, true cretinism is more frequently encountered.

The morphology and function of the type of goiter that occurs in Russia resembles that of the goiter found in Southern and Central Germany. The most characteristic form of the goiter in Russia is the macrofollicular colloid goiter

with epithelial proliferation, with or without small colloid nodules. This form predominates in the endemic regions of the plains and in mild endemic regions of the mountains. In so-called goiter-free regions this colloid goiter is often associated with clinical symptoms of severe or mild grades of exophthalmic goiter.

In Arndt's material twenty-eight different races or nationalities are represented. He denies a true race disposition for goiter and believes that exogenic factors, especially nutrition and standard of living, play a much more important rôle in the etiology of goiter.

C. A. HELLWIG.

ARTIFICIAL PRODUCTION OF CORPUS LUTEUM BY INJECTION OF PLACENTAL SUBSTANCE. M. MURATA and K. ADACHI, Ztschr. f. Geburtsh. u. Gynäk. **92**:45, 1927.

The injection of an emulsion or extract of placenta from an early period of pregnancy, of hydatiform mole and of malignant chorio-epithelioma produces corpora lutea in rabbits. There are two types of corpora lutea, those originating from ruptured follicles and those from nonruptured ones. The efficacious substance is derived from the chorionic epithelium. This incretion apparently prevents the regression of the corpus luteum during pregnancy. The corpus luteum producing quality of the incretion, as observed in the test animal, is an expression of the same effect as is shown in its specific stimulating influence on the granulosa cells. These are transformed into lutein cells simultaneously with a change of the follicle into a corpus luteum. The transformation of a follicle into a corpus luteum and the ovulation are, therefore, two processes independent of each other. The polycystic degeneration of the ovaries as observed in hydatiform mole and chorio-epithelioma can be compared with the artificial production of corpora lutea.

W. C. HUEPER.

THE EFFECT OF THE SERUM OF PREGNANT WOMEN ON THE MALE SEXUAL ORGANS. E. FELS, Ztschr. f. Geburtsh. u. Gynäk. **93**:50, 1928.

The serum of pregnant women was injected into male mice. The female sexual hormone impairs the growth of the heterologous sexual glands. This antagonistic effect is apparent only in organs still in the developmental stage. It is not effective in adult organs. The serum activates also the development of the seminal vesicles, prostate and penis by the large amount of the anterior pituitary hormone present in the serum. This acts directly on the interstitial cells of the testicle which proliferate. Therefore as producers of the male sexual hormone the interstitial cells play an important rôle.

W. C. HUEPER.

Pathologic Anatomy

HEMORRHAGES FROM LACERATIONS OF THE CARDIAC ORIFICE OF THE STOMACH DUE TO VOMITING. G. K. MALLORY and S. WEISS, Am. J. M. Sc. **178**:506, 1929.

Severe, occasionally fatal, gastric hemorrhage with hematemesis may occur after periodic alcoholic debauches. Acute lacerations with ulcerations at the cardiac opening of the stomach were the sources of such fatal gastric hemorrhages in three patients here described. Lacerations of the cardiac opening of the stomach leading to massive hemorrhage may also develop in other diseases, for example, pernicious vomiting of pregnancy and malignant neoplasms, if associated with persistent nausea, retching and vomiting. The responsible etiologic factors producing the described lacerations are: pressure changes in the stomach during a disturbed mechanism of vomiting, and continuous regurgitation of gastric juice over the mucosa of the cardia. The local irritant and astringent effect of alcohol as well as the nature of vascularity of the mucosa involved are also

predisposing factors. In the presence of obscure gastric hemorrhage, or of hematemesis, lesions of the cardiac opening of the stomach should be thought of, and in case surgical interference is undertaken the folds of the cardia should be explored carefully.

AUTHORS' SUMMARY.

SPONTANEOUS INTRACRANIAL HEMORRHAGE FROM A VASCULAR TUMOR. R. J. REITZEL and P. BRINDLEY, Am. J. M. Sc. **178**:689, 1929.

A case of fatal intracranial hemorrhage from a cerebral vascular telangiectasia in a young man is reported, with observations at autopsy, and a brief review and discussion of the literature. A vascular tumor should be borne in mind as a possible source of intracranial hemorrhage when such hemorrhage occurs in a previously healthy young adult.

AUTHORS' SUMMARY.

CEREBRAL HEMORRHAGE FROM VENOUS AND CAPILLARY STASIS. STANLEY COBB and JOHN P. HUBBARD, Am. J. M. Sc. **178**:693, 1929.

Five cases are presented which at autopsy showed hemorrhage into the parenchyma of the brain. In all of these the extravasation came from the veins and capillaries; there is no evidence that blood escaped from arteries. The mechanical factor common to all these cases is venous stasis; the common chemical factor is asphyxia. It is pointed out that widespread lesions can be caused by these two factors, the unit lesion being a petechial extravasation, but large lesions are formed by coalescence, and frank venous hemorrhage may be found. Such cases are more common than is generally supposed.

AUTHORS' SUMMARY.

OBLITERATIVE CHANGES OF THE PULMONARY LYMPH VESSELS IN TUBERCULOSIS. J. DWIGHT DAVIS, Am. Rev. Tuberc. **19**:595, 1929.

A thrombus occasionally forms in lymph vessels which drain the area of a tuberculous focus in the lungs. It is due to sensitivity of the endothelial lining of the vessel, and results in proliferation of the endothelial cells with a formation of fibroblasts. The formation of the thrombus continues until the lumen of the lymph vessel is densely packed with these fibroblastic cells. Following this and without organization, degenerative or retrogressive changes occur. Usually these changes appear as a vacuolization and replacement of the cell by fibro-elastic tissue, or by a transformation of the fibroblasts into hyaline material. Canalization of the occluding mass also is seen. This proliferation is entirely separate from the formation of tubercles within the lymph vessels, which also may occur. The type of proliferation, although it is due to the stimulation of tuberculous products, is not distinctive, and in itself it would not be known to be of tuberculous origin.

H. J. CORPER.

REPORT OF FIFTY AUTOPSIES IN WHICH THE PRIMARY CAUSE OF DEATH WAS PULMONARY TUBERCULOSIS. AUDLEY O. SANDERS, Am. Rev. Tuberc. **20**: 128, 1929.

A summary report of fifty autopsies is presented, only those cases being included in which gross tuberculous pulmonary lesions were extensive and in which there was no doubt that the tuberculous lesions were the primary cause of death. Forty-five of the patients were white and five colored. The ages ranged from 26 to 62 years. The cases included one of tuberculosis of the bones and joints, two of chronic purulent otitis media and eighteen of laryngeal tuberculosis. No gross abnormality of the thyroid was noted in any case. In forty-one cases there were bilateral pleural adhesions, and in nineteen, pneumothorax, which in ten instances was therapeutic. All but three of the fifty cases showed

gross bilateral pulmonary lesions, thirty-two having more or less extensive bilateral cavitation, sixteen unilateral cavitation and two no cavities. In general, the size of the heart and condition of the cardiac musculature corresponded closely to the degree of general nutrition and the muscle tone. In twenty-two cases there were slight atheromatous changes in the aorta, while in three there was marked atheroma. In sixteen cases the peritoneal cavity contained fluid; in nine of them there was pleural effusion, the effusion being pericardial in five. No definite tubercles or caseous nodules were found in the livers, but there were two cases of cirrhosis of the liver, in both of which there was an alcoholic history. No gross or evident pathologic change was noted in the gallbladder in any case. No pathologic change was noted in the pancreas in any case. In nine cases there were definite changes in the spleen and in one case the left suprarenal body was enlarged. In six cases tuberculous lesions were found in the kidney; in three instances they were bilateral. Extensive involvement of the bladder was observed in two cases and anomalous kidneys were found in one. Of nine cases in which extensive lesions of both the small and the large intestine, were observed, no note was made in the clinical records of any bowel symptoms except moderate constipation in three cases, abdominal distress alone in one case and abdominal distress together with diarrhea in five cases. Of ten cases with lesions only in the small bowel no abdominal symptoms were recorded clinically in four cases, moderate constipation in two and abdominal distress with diarrhea in four. Of two cases with lesions only in the cecum and the colon, no mention was made of abdominal symptoms in one and diarrhea was noted in the other. Of the fifty cases, the clinical records reported thirteen with notable diarrhea; tuberculous intestinal lesions were found in ten of these thirteen at autopsy.

H. J. CORPER.

HISTOLOGY OF HEALED PRIMARY TUBERCULOUS LESIONS OF THE LUNG. R. H. JAFFÉ and S. A. LEVINSON, Am. Rev. Tuberc. **20**:214, 1929.

The tendency of the tuberculous primary lesion of the lung to become replaced by bone is explained on the basis of an irritating action of the calcified center on the specific capsule. This irritating action leads to an ingrowth of granulation tissue, which, bathed with tissue fluids saturated with calcium salts, undergoes osseous metaplasia. This metaplasia is characteristic of the primary lesion and is perhaps due to its peculiar histogenesis. It is seldom observed in the calcified and encapsulated tubercles of regional lymph nodes and of reinfections.

H. J. CORPER.

BENIGN TUMORS OF THE FEMALE BREAST. G. VAN S. SMITH and G. A. MARKS, Surg. Gynec. Obst. **49**:316, 1929.

A clinical and pathologic study of 201 cases of benign tumors of the breast led to the following conclusions:

Periductal or intracanalicular adenofibromas were present in 28.3 per cent of cases, occurring usually in patients under 30 years of age; 7 per cent were associated with sarcoma and 13 per cent were bilateral.

Fibrocystadenomas or fibroadenomas occurred in 7.9 per cent of the patients, all of whom were under 35 years of age; 14.2 per cent had a discharge from the nipple, and 28 per cent were associated with carcinoma.

Papillary cystadenomas occurred in 6.9 per cent.

Chronic cystic mastitis occurred in 56.7 per cent of the specimens. The patients were all over 35 years of age; 8.8 per cent had a discharge from the nipple, and 3.4 per cent were bilateral.

Abnormal menstruation occurred in 42.3 per cent of those who had not passed the menopause.

RICHARD A. LIFVENDAHL.

THE HISTOGENESIS OF ADENOMYOSITIS. G. L. MOENCH, Surg. Gynec. Obst. **49**:332, 1929.

The author is of the opinion that many of the areas containing glands which are surrounded by an endometrial stroma-like tissue are not necessarily evidences of endometriosis. In some instances he regards them as serous peritoneal cysts surrounded by an inflammatory reaction, whereas others are normal occurring embryonal tubules. Those cysts that are located in the lower part of the vagina and the hymen he regards as being derived from the wolffian duct. The various theories which have been propounded to explain these variously located adenomyositic lesions are discussed.

RICHARD A. LIFVENDAHL.

DISLOCATION OF THE TESTIS. E. P. ALYEA, Surg. Gynec. Obst. **49**:600, 1929.

Traumatic dislocation of the normally situated testis is dependent on three factors: (1) anatomic factors such as, an atrophic testis, relaxation of the external inguinal ring or the presence of an inguinal hernia; (2) obstruction at the scrotal neck, inguinal or perineal regions, and (3) the direction and the force of the blow. Thus, if a subcutaneous course is taken, the organ may come to rest in the pubic, superficial inguinal, penile, crural or the perineal regions. If the path is through the inguinal canal, it may lodge here, intra-abdominally, or in the femoral canal. To these two groups, the author adds a third to which he applies the term "compound dislocation of the testis," in which the testis is forced through a tear in the scrotum. He reports one case of this type and quotes three similar instances from various authors. The writer's other patient presented a very unusual condition in that the testicle was located over the fascia in the region of the apex of the femoral triangle and 12 cm. below the external inguinal ring. The twenty-three original occurrences of traumatic dislocation of the testis collected from the literature, since 1800, is reviewed in detail.

RICHARD A. LIFVENDAHL.

KRUKENBERG TUMOR OF THE OVARY. R. FALLAS, Surg. Gynec. Obst. **49**: 638, 1929.

The typical gross and microscopic picture of two cases of Krukenberg tumor of the ovaries is added to the literature. In one, exploration of the upper part of the abdomen revealed an indurated area in the stomach. The second is unusual in that no primary source in the gastro-intestinal tract could be determined, clinically; however, cells characteristic of these tumors were found in a rapidly growing tumor of the breast in this patient. The possibility of the ovarian growths being secondary to the neoplasm of the breast is considered. Nothing new is added to the previous theories as to the path of transmission of these tumor cells to the ovaries.

RICHARD A. LIFVENDAHL.

EMBRYONAL ADENOMYOSARCOMA OF THE KIDNEY IN AN ADULT. E. F. KILBANE and C. W. LESTER, Surg. Gynec. Obst. **49**:710, 1929.

Embryonal adenomyosarcoma (Wilm's tumor, embryonal adenosarcoma or embryonal sarcoma) of the kidney occurs usually during infancy, but the writers report this type of tumor in a man, aged 48, which makes the sixteenth case on record that has been reported in adults. Grossly, the tumor was characteristic in that it was large, being 16 cm. in diameter, weighed 980 Gm., was sharply separated from the remaining renal tissue by a connective tissue capsule, and was centrally necrotic. The connective tissue elements had the appearance of a rapidly growing sarcoma; the epithelial structures were composed of malignant appearing oval cells arranged in cords and tufts or cuboidal cells assuming a tubular fashion, and the muscle fibers were in the form of slender strands. No

striated muscle, bone or cartilage were found, as has been previously recorded in these neoplasms. Following the removal of this kidney, there were local subcutaneous recurrences of the epithelial elements in a sinus through the skin at the operative site.

RICHARD A. LIFVENDAHL.

ANATOMIC OBSERVATIONS IN CHOKED DISK FROM MYELOGENOUS LEUKEMIA. P. A. JAENSCH, Arch. f. Ophth. **122**:618, 1929.

Changes in the eye as a result of leukemia are well known. The anatomic observations, however, have been less frequently published. The author has seen five myelogenous leukemias and one aleukemic lymphadenosis with retinal hemorrhage.

White spots and frequently prominent hemorrhages, discoloration of the fundus to orange-red, distention of the veins and changes in color of the blood vessels have been described.

In addition to normal observations, the disk showed every transition stage from blurring of the borders and neuritis of the optic nerve to formation of a high grade choked disk. These observations are true for both myelogenous and lymphatic leukemia. The choroid, however, shows distinguishing features for the two conditions. In lymphatic leukemia there is a diffuse infiltration of the stroma; the choroid may be thickened to four or five times its normal condition. Blood vessels are usually empty and of normal caliber; sometimes their walls are infiltrated.

In myelogenous leukemia, the similarly distended choroid is due to an engorgement of the blood vessels, but in the choroid stroma, which is usually very meager, no cells can be demonstrated. The excessive number of intravascular cells may be so great that the vascular walls cannot be recognized by the usual staining method, and only a demonstration of elastic fibers shows that all the white blood cells lie within the vessels.

The author gives a full description of observations in the myelogenous leukemia (secondary myeloblastic leukemia) of a boy, aged 9 years, in whom both bulbi showed signs of a high grade choked disk: The dilated veins and capillaries of the entire uvea and of the retina and disk as well as of the optic nerve were engorged. Cells showing fatty degeneration were found in two places and were thought to be derived from the histiocytes and not connected with the basic disease.

CHARLES WEISS.

GENERALIZED MILIARY LYMPHOGRANULOMATOSIS. O. GSELL, Beitr. z. path. Anat. u. z. allg. Path. **81**:426, 1928.

A man, aged 53, had become gradually and progressively weaker and emaciated during the year preceding his death. Two weeks before death he became jaundiced. There had been no local symptoms, and on admission to the hospital in a moribund condition no external changes except jaundice and emaciation were to be noted. Records of the blood picture and of the temperature were wanting. At necropsy the lungs were studded by innumerable small nodules, which suggested miliary tuberculosis. The liver was enlarged to 3.5 Kg. and contained multiple larger, pale nodules, up to the size of a fist, which suggested metastatic tumors. In the moderately enlarged spleen were nodules larger than those in the lung and smaller than those in the liver, together with diffuse changes, which suggested lymphogranulomatosis. On microscopic examination, the nodules of the lung were found to be composed of lymphocytes, fibroblasts, proliferated endothelium, a few eosinophils and large cells of the Sternberg-Dorothy Reed type, all embedded in a fibrillar reticulum. Histologically, the miliary nodules were typical lesions of lymphogranulomatosis. Amyloid material was present in them in the form of streaks and masses. Microscopic lesions of the same kind were present everywhere in all the tissues except those of the myocardium, brain, suprarenal glands and prostate. The larger nodules were of the same general histologic structure as the miliary ones, except that they contained a larger amount of collagenous tissue.

At the periphery of the large nodules the tissue was identical with that of the miliary and microscopic lesions. The periportal lymph nodes were enlarged. The case is considered one of lymphogranulomatosis with unusually generalized distribution, involving the lymphoid and areolar mesenchymal tissues throughout the body. Amyloid deposition in lymphogranulomatous lesions has been previously described, but occurs infrequently.

O. T. SCHULTZ.

PARIETAL ENDOCARDIAL SCLEROSIS. A. BÖGER, Beitr. z. path. Anat. u. z. allg. Path. **81**:441, 1929.

On the basis of selected examples described in detail, Böger discusses the nature and origin of sclerosis or thickening of the parietal endocardium, so-called chronic parietal endocarditis. According to its gross characteristics, the condition discussed may be divided into the diffuse and the localized, and according to its etiology into the inflammatory and noninflammatory. The diffuse thickening of the parietal endocardium involves an entire chamber or more than one chamber. It is not inflammatory in nature and is the result of the reaction of the elastic and connective tissue of the endocardium to dilatation of the chamber. It is usually not associated with inflammatory changes in the valves, although such association may be noted if the valvular involvement has led to dilatation of a cardiac chamber. Increase of both elastic and collagenous tissue elements characterizes the process histologically. Localized areas of endocardial thickening are usually inflammatory in origin and are associated with inflammatory changes in the wall of the heart. Most often the localized process is the result of contact of the parietal endocardium with an inflamed valve, most commonly with the posterior surface of the aortic segment of the mitral valve. Involvement of a localized area of parietal endocardium by direct continuation of an inflammatory process from the myocardium was not observed in Böger's series of cases, but the possibility of its occurrence is granted. The purely functional, noninflammatory origin of localized endocardial thickening is denied by the author, who also denies the formation of endocardial pockets as a primary process. Such pockets are the result of the action of the blood stream on the ridges of localized areas of endocardial thickening.

O. T. SCHULTZ.

NATURE OF TUBERCULOUS CASEATION. H. SCHLEUSSING, Beitr. z. path. Anat. u. z. allg. Path. **81**:473, 1929.

Coagulation necrosis was first described by Weigert in 1879 and repeated observations on it as a pathologic process are to be found in his later writings, especially those to 1891. Tuberculous caseation was considered by Weigert a form of coagulation necrosis, that is, a form of sudden tissue death characterized by coagulation of cellular protoplasm, which need not necessarily show any great pathologic alteration before it undergoes coagulation. Schleussing claims that, although later writers still include tuberculous caseation among the examples of coagulation necrosis, their morphologic descriptions are not entirely in accord with those of Weigert and emphasize the occurrence of cellular degenerative changes, such as cloudy swelling, fatty change and nuclear fragmentation as necessary antecedents to caseation. Believing that such views would make caseation a process different from the coagulation necrosis described by Weigert, he made a morphologic study of caseating tuberculous lesions and attempts an interpretation of the nature of the process. Although cellular degenerative changes are not unusual in such lesions, their chief morphologic characteristic is, as was maintained by Weigert, the retention of cell contours and of the outlines of tissue elements, especially swollen collagen fibers, in the dead tissue. The sudden tissue death is the result of the action of an excessive dose of or excessively strong toxin, or of greater susceptibility of the tissue to the action of toxic material. The essential factor in the coagulation is the presence in the tissues, both within and outside the tissue elements, of an increased amount of fluid capable of coagulation. Caseation differs from ordinary sudden necrosis, such as may sometimes

also be caused by the tubercle bacillus, in that it occurs in an inflammatory tissue. Huebschmann, Medlar and others considered ferments liberated by leukocytes important in the coagulation. Schleussing was not able to convince himself of the importance of this factor. He considers the inflammatory edema of an allergically hypersusceptible tissue as the underlying phenomenon of tuberculous caseation, which is a typical coagulation necrosis as defined by Weigert.

O. T. SCHULTZ.

THE ALVEOLAR EPITHELIUM OF THE LUNG. G. SEEMANN, Beitr. z. path. Anat. u. z. allg. Path. **81**:508, 1929.

One of the most actively discussed problems in contemporary scientific medical literature relates to the origin and nature of the phagocytic cells of the lung. Three views are extant; that which derives these cells from the respiratory alveolar epithelium, that which derives them from the capillary endothelium and that which derives them from the septal histiocytes. In the course of the controversy, there have arisen some investigators who deny entirely the existence of any epithelial lining within the alveoli of the lung. This view, however, is not a new one; it is the original view of the structure of the lung, as championed by Zenker and Villemin, who considered the lung a mesenchymal structure devoid of epithelium except in the bronchi. It was Kölliker, who, in the early eighties of the past century, as the result of intrabronchial silvering, described an alveolar lining composed of smaller nucleated cells separated by flattened nonnucleated plates. This specific respiratory epithelium was entodermal in origin. Aschoff and his school have taken a stand for the origin of the phagocytes of the lung from the alveolar epithelium, and Seeman, in a previous paper from Aschoff's laboratory, concluded, from vital staining methods, that the phagocytes of the lung are derived from alveolar epithelium but constitute a biologic cell type *sui generis*. The present work is a histologic study undertaken to determine the existence of alveolar epithelium. The methods used were silvering of fresh lung tissue by intrabronchial injection of silver nitrate solution; intracapillary silvering of the tissue by the injection of silver nitrate solution into the pulmonary artery; demonstration of the reticulum of the septums, and histologic study of the embryonic lung. As the result of his study, Seemann claims that intrabronchial silvering stains electively and specifically the alveolar epithelium. Intracapillary silvering stains electively the capillary endothelium, and the two methods permit a sharp topographic distinction between capillary endothelium and alveolar epithelium. The capillary endothelium is covered by a fenestrated membrane which has the staining properties of reticulum. The epithelium does not form a complete layer. When it is present, the epithelium is separated from the endothelium by the reticulum membrane. The nonnucleated respiratory epithelial plates, described by Kölliker, do not exist; in areas in which epithelium is not present, the reticulum membrane is devoid of covering. Seemann claims that intrabronchial silvering after irritation of the lung brings out clearly the epithelial origin of the phagocytic cells. He denies that the alveolar epithelium present has any respiratory function, but ascribes to it a protective function, manifested by its great phagocytic activity. In the embryologic development of the lung, the transformation of a complete lining layer of cuboidal alveolar epithelium into an incomplete lining formed of separated flattened epithelial cells can be followed.

O. T. SCHULTZ.

FATE OF INJECTED HOMOLOGOUS LEUKOCYTES. E. CHRISTELLER and G. EISNER, Beitr. z. path. Anat. u. z. allg. Path. **81**:524, 1929.

This is what was apparently to be the first report of a line of experimental investigation on which Christeller was engaged at the time of his sudden death. The origin of inflammatory exudate cells, ascribed to the blood stream by Cohnheim, has aroused renewed interest because of recent work which derives such cells from local tissue elements, the adventitial cells of Marchand, the slumber

cells of Grawitz, the tissue histiocytes and the tissue lymphocytes. Christeller and Eisner hoped to approach the problem of the participation of circulating leukocytes in inflammatory exudate by using homologous leukocytes which they could recognize in the vessels and tissues of a recipient animal. Sterile subcutaneous abscesses were produced in dogs by the injection of 1 cc. of turpentine. Two days later, 10 cc. of a 2 per cent solution of trypan blue was injected directly into the abscess. Fifteen minutes later the pus, which consisted largely of stained leukocytes, was withdrawn, washed in sodium chlorid-sodium citrate solution, suspended in this solution and used for intra-arterial or intravenous injection into normal recipient animals. The animals were killed from two to seven minutes after injection of the leukocytes, and the tissues were examined microscopically to determine the distribution of the marked leukocytes. In general it was found that many of the leukocytes tended to attach themselves to the endothelium of the capillaries of the territory of the vessel selected for injection. Many, however, entered the general circulation. These were quantitatively removed from the blood by the capillaries of the lung in the first passage of the blood through the lungs. The removal of damaged cells from the blood is considered an important function of the pulmonary capillary circulation. When marked leukocytes were injected into recipient animals in which sterile abscesses were caused by turpentine, none of the stained leukocytes could be found in the local inflammatory exudate.

O. T. SCHULTZ.

CHANGES IN THE VERTEBRAL COLUMN OF THE NEW-BORN INFANT IN CHONDRODYSTROPHIA. F. KNÖTZKE, Beitr. z. path. Anat. u. z. allg. Path. **81**:547, 1929.

Older writers, on the basis of gross examination, described lordosis and kyphosis as the only changes shown by the vertebral column in chondrodystrophia. Later observers have reported histologic alterations in the bodies and laminae of the vertebrae almost as marked as those of the long bones. In spite of such alterations the vertebral column, except for such lordosis or kyphosis as may be present, is usually as long as that of a normal adult. Seeking to find an explanation of this fact, Knötzke made a comparative study of the long bones, the vertebral column and the cranial bones of a normal, full-term, new-born infant and of a full-term, new-born chondrodystrophic infant. He concludes that chondrodystrophia is a disturbance, not of cartilage formation, but of enchondral bone formation. The changes characteristic of the disease are therefore greatest in the bones laid down primarily as cartilage, namely, the long bones and those of the base of the skull, and least in other bones, that is, the vertebral bodies and the bones of the cranial vault. The vertebral laminae are affected in the same way and to the same degree as are the long bones, shortening of the laminae resulting in a decrease of the anteroposterior diameter of the spinal canal. The thickening of the periosteum at the epiphyses of the long bones is considered probably compensatory in nature. Interference with the development of the ossification centers of the vertebral bodies and fibrosis of the medullary canals occur only in the direction of the long axis of the notochord, not in all directions as in the long bones, hence the degree of deformation of the vertebral bodies is relatively slight. In the epiphyses of the long bones, as in the bodies of the vertebrae, endochondral bone formation runs a more normal course than in the shafts of the long bones; the epiphyses therefore reveal a relatively lesser decrease in size than do the shafts of the long bones.

O. T. SCHULTZ.

HAIRY POLYP OF PHARYNX. A. FELLER, Beitr. z. path. Anat. u. z. allg. Path. **81**:658, 1929.

Feller describes a pharyngeal hairy polyp obtained from a stillborn, full term infant. The tumor was the size of a hen's egg and was attached at the posterior portion of the hard palate and the vault of the pharynx by a broad base. It was

covered by skin and by a growth of fine hairs. On microscopic examination, it was found to contain well differentiated derivatives of the three germinal layers. The structure was organoid in character, a teratoma and not an embryoma. The author places the tumor in group III of Schwalbe's classification of the epignathi. In accordance with this classification, the author derives the structure from a totipotent misplaced blastomere.

O. T. SCHULTZ.

BILATERAL DUCTUS ARTERIOSUS. G. SALZER, Beitr. z. path. Anat. u. z. allg. Path. **81**:671, 1929.

In an infant, a girl, who died at the age of 7 weeks, a diagnosis of congenital heart disease had been made shortly after birth. She had been cyanotic from birth. The patient was admitted to the hospital at the age of 1 month for infectious pemphigus. The area of cardiac dulness was increased, and the second aortic sound was intensified. The cyanosis became gradually more intense, and the child died in spite of improvement in the infectious condition of the skin. The clinical diagnosis was congenital heart disease, probably transposition of the arterial trunks. At necropsy the heart was found greatly enlarged. It consisted of a single ventricular chamber and a single auricular chamber. Nothing suggestive of a rudimentary ventricular septum could be found. In the auricle was a small ridge which was thought to be probably part of the lower boundary of the foramen ovale. The trunk of the pulmonary artery was obliterated. A persistent ductus botalli united the right pulmonary artery with the arch of the aorta, and a similar structure united the left pulmonary artery with the aorta at the level of origin of the left subclavian artery. The anomaly is considered a maldevelopment resulting from inhibition of growth (Hemmungsmissbildung), the inhibition manifesting itself in failure of the formation of the cardiac septums and in failure of the normal regression of the gill cleft arteries.

O. T. SCHULTZ.

HISTOGENESIS OF THE INFLAMMATORY REACTION. A. A. MAXIMOW, Beitr. z. path. Anat. u. z. allg. Path. **82**:1, 1929.

This paper, the last from the pen of Maximow, restates the results of a line of work on which he had been engaged since 1902 and reiterates the views held as the result of that work. Although it takes into consideration all of his older work relating to the histogenesis of the inflammatory reaction, it is based also on new experimental studies of the inflammatory reaction of the subcutaneous tissue of the rat, undertaken with the aim of testing the validity of the contentions of the von Möllendorfs relative to the origin of inflammatory exudate cells. In the normal subcutaneous areolar tissue of the rat, Maximow recognizes, in addition to mast cells and fat cells, only three types of cells, fibroblasts, resting wandering cells or histiocytes and eosinophilous leukocytes, the latter being distinct from the granular leukocytes of the blood. Under the influence of an inflammatory stimulus, granular leukocytes, lymphocytes and monocytes invade the tissue from the blood stream, and the local histiocytes become transformed into ameboid, phagocytic polyblasts, as do also the lymphocytes which have emigrated from the blood. The origin, transformations and fate of the hematogenous granular leukocytes, the fibroblasts and the polyblasts are described, greatest attention being given to the polyblasts. The contribution is an evaluation of results obtained by the use of careful histologic technic and of conclusions reached by the mode of thought of the anatomist, as compared with the results obtained by the vital, supravital, physical, chemical and physicochemical methods of the modern experimental pathologist and of the mode of thought of "Pathologen und Kliniker." It is a scathing criticism of what Maximow considered to be poor technical methods. It is a caustic criticism of the conclusions reached by others by means of improper technic. It is a sarcastic criticism of the work of those who have apparently entirely neglected or overlooked his own previous work. It is a polemic against von Möllendorf and his school, who ascribe

embryonic multiple potencies to what they term the fibroblastic syncytium, which may give rise to all the various types of cells present in an inflamed tissue. It is the cry of a man who felt that his life's work had not been appreciated. This feeling is suggested in Maximow's maximum in his opening paragraph, "Es wird aber heutzutage in der Biologie und Medizin so viel geschrieben und so wenig gelesen," and is apparent in the frequent references to his own work, knowledge of which should have prevented the appearance of much that has been published. Maximow was a master of several languages and could express himself equally well in all of them; he seemed to know the idiom of the language in which he wrote. Certainly no German ever used his own language more bitingly and more incisively, yet withal more precisely, than did Maximow in his last contribution. It is published as left by him, in the form of his first draft. Perhaps if he had lived he would have softened its tone somewhat, which would have been unfortunate. It is an excellent summary of Maximow's work and views in a field to which he devoted much of the energy of a lifetime. The article opens with an introduction by Maximow's pupil, colleague and friend, William Bloom, who describes the circumstances under which the manuscript was prepared. It closes with a short paragraph by Maximow's colleague and friend, Ludwig Aschoff, who regrets that Maximow had the feeling that his own work was not properly appreciated by German pathologists. There are thirteen plates excellently reproduced in halftone.

O. T. SCHULTZ.

SENILE FIBROSIS. L. MÜLLER, Beitr. z. path. Anat. u. z. allg. Path. **82**:57, 1929.

Senile fibrosis is defined by Müller as an increase in the fibrous framework of the parenchymatous organs of the body. If such a process occurs it must be a physiologic manifestation of advancing age, and all other factors such as inflammation, degeneration and venous stasis must be excluded. To determine whether there actually is such a thing as senile fibrosis, he made a comparative study of the framework of various organs and tissues of persons of various ages. Paraffin sections of uniform thickness were subjected to trypsin digestion and were stained and the framework was studied. As the result of such study he divides the supporting framework of tissues and organs into a reticulum fiber system, which occurs in the spleen, lymph nodes and liver, and a basal membrane system, which occurs in adipose tissue, skeletal muscle, myocardium, suprarenal glands, thyroid gland, hypophysis, kidney and lung. The first, which is composed of a meshwork of fibrils, corresponds to what has been called the reticulum of the organs in which it occurs. The second, composed of a meshwork of flattened, membrane-like fibers, is more extensive than the gitter fibril system of the tissues in which it occurs. The framework is considered a homogeneous transformation of the embryonic mesenchymal ground substance. Collagen is not present in it, except in the broader supporting bands which separate lobules or lobes and in the capsules of organs. The framework described by Müller does not increase with age, either relatively or absolutely. On the contrary, with age there occurs a progressive decrease in the supporting framework which runs parallel with the disappearance of parenchymatous elements. A relative thickening of the capsule and septums occurs as the result of senile atrophy of the organs. Müller concludes that there is no true senile fibrosis.

O. T. SCHULTZ.

TRANSFORMATION OF EMIGRATED LYMPHOCYTES INTO POLYBLASTS IN THE INFLAMMATORY REACTION. A. KARMALLY, Beitr. z. path. Anat. u. z. allg. Path. **82**:92, 1929.

In the number of Ziegler's *Beiträge* that contains Maximow's last word on the polyblast theory, appears this brief report, based on work done in Aschoff's laboratory, of results which are interpreted as diametrically opposed to Maximow's repeated assertion that emigrated lymphocytes become transformed into polyblasts in the field of inflammation. Before reading this article, one should

read, in the original, what Maximow has to say about technical methods. Karmally believes that many of the emigrated mononuclear blood cells that Maximow held to be lymphocytes were probably monocytes. He substantiated this belief by the use of the supravital staining method, by means of which he claims that it is easy to distinguish between the lymphocytes and the monocytes of the rat's blood. He set up inflammatory reactions in the skin and in the peritoneal cavity of normal rats and of rats which had been treated with benzene or with the roentgen rays. In normal animals, emigrated leukocytes and monocytes predominate in the inflammatory area; the histiocytes take part in the reaction only secondarily and later. In animals treated with benzene, emigration of leukocytes and monocytes is slight, and the histiocytic reaction predominates. The cellular composition of the inflammatory tissue of rats treated with the roentgen rays is intermediate between the other two. In the inflammatory peritoneal exudate of rats receiving preliminary treatment with benzene or the roentgen rays, leukocytes predominate and lymphocytic emigration is slight. Gradually the monocytes (histiocytes) become more numerous and the lymphocytes become correspondingly fewer. In preparations of the blood and peritoneal exudate that were incubated, a transformation of lymphocytes into monocytes could not be observed. Karmally admits the transformation of monocytes into polyblasts, but denies the transformation of lymphocytes into monocytes or polyblasts.

O. T. SCHULTZ.

SIGNIFICANCE OF FINELY GRANULAR CALCIUM IN THE ARTERIAL MEDIA. E. FARKAS and P. FASAL, Beitr. z. path. Anat. u. z. allg. Path. **82**:102, 1929.

A systematic study of the presence of finely granular calcium in the media of the arterial system was undertaken by the authors for the purpose of determining a possible relation of this process to arteriosclerosis and atherosclerosis. Deposition of finely granular calcium in the media was encountered so frequently in persons of 20 years of age and over as to appear almost physiologic. The quantity of the deposit increases with age. It bears no apparent relationship to any disease. In arteries that showed no change other than the presence of finely granular calcium in the media, the percentage distribution of the process for the various arteries examined was as follows: coronary, 9 per cent; renal, 11 per cent; radial, 21 per cent; femoral, 39 per cent; aorta, 83 per cent. In cases with fibrous thickening of the intima (arteriosclerosis), the percentage distribution was as follows: coronary, 4 per cent; renal, 20 per cent; radial, 65 per cent; femoral, 75 per cent; aorta, 83 per cent. In cases with true medial calcification (atherosclerosis), the figures were as follows: coronary, 25 per cent; renal, 35 per cent; radial, 75 per cent; femoral, 82 per cent; aorta, 95 per cent. Although the process is relatively somewhat more frequent in arteries which are the seat of atherosclerosis, the authors do not think that the presence of finely granular calcium in the media bears any causal relationship to atherosclerosis. The process is the result of the functional demands made on the vessel wall and is the result of physiologic wear and tear of the arterial tissues.

O. T. SCHULTZ.

EFFECT OF EXPERIMENTAL OCCLUSION OF THE RENAL VEIN. R. NISHII, Beitr. z. path. Anat. u. z. allg. Path. **82**:112, 1929.

Thrombotic occlusion of the renal vein in the human being has variable effects, sometimes slight, sometimes grave, depending on the rapidity with which the occlusion occurs. If this is gradual, a good collateral circulation is developed and the effects are slight. It has been claimed by several workers that a collateral circulation is not readily developed in the rabbit after occlusion of the renal vein, this animal differing in this respect from the human being and the dog. Nishii repeated such experiments and attempted also to bring about a gradual narrowing of the vein with final complete occlusion. Sudden complete stoppage of the venous outflow led to necrosis of the kidney that was complete except for small areas near the hilum. The kidney underwent fibrous contraction, calcification and even

bone formation. If the hindrance to the outflow was more gradually brought about, little change occurred so long as any outflow was possible. The final complete occlusion of a renal vein which had been narrowed by previous procedures resulted in necrosis of the kidney. In one such experiment, however, a collateral circulation developed and little damage was done to the kidney by the final closure of the vein. Nishii concludes, in agreement with others, that the degree of injury following occlusion of the renal vein depends on the degree to which a collateral circulation is developed, and that in the rabbit an adequate collateral circulation is not readily formed.

O. T. SCHULTZ.

ARTERIAL CHANGES IN SPONTANEOUS GANGRENE. K. SPONHEIMER, Beitr. z. path. Anat. u. z. allg. Path. **82**:122, 1929.

The author criticizes the modern tendency in pathology which makes functional changes predominant and morphologic alterations secondary. He feels that this tendency manifests itself in discussions relating to the pathology of the vascular system, in which vasomotor changes are held responsible by some for all the anatomic alterations encountered. As a contribution to the subject he presents two cases of gangrene of an extremity. In the first case, that of a man, aged 28, the vascular lesion was a typical thrombo-angiitis obliterans. The author accepts and presents Buerger's conceptions of this disease in great detail. The second case was one of gangrene of the left foot in a man, aged 38, with recurrent polyarthritis and a fresh rheumatic endocarditis on the basis of an older rheumatic valvular lesion. The vessels of the extremities, both upper and lower, were greatly thickened and their lumen was diminished. No details of microscopic observations in the second case are presented.

O. T. SCHULTZ.

THE PRESENCE OF FIBRIN IN THE TUBERCLE. K. EVELBAUER, Beitr. z. path. Anat. u. z. allg. Path. **82**:141, 1929.

Following a general discussion of exudative inflammatory reactions in the tubercle, the author describes the localization and morphology of fibrin in the early tubercles present in the liver and spleen in three cases of chronic pulmonary tuberculosis with cavity formation and caseous pneumonia. He found the Eppinger method for demonstrating bile canaliculi the best staining method for fibrin in the tissues. He made two slight modifications in the Eppinger method. After mordanting of the block of tissue, sections were cut on the freezing microtome instead of in celloidin, and the hematoxylin solution was allowed to act for twenty-four hours at incubator temperature instead of from one-half to several hours as in the original method.

O. T. SCHULTZ.

CARDIAC MALDEVELOPMENTS IN ADULTS. H. G. SCHRAMM, Beitr. z. path. Anat. u. z. allg. Path. **82**:153, 1929.

Two cardiac anomalies of types usually considered incompatible with a long duration of life are described. In a man, aged 48, the aorta arose to the right of and behind the pulmonary artery, being situated between the latter and the right auricular appendage. There was a subaortic defect of the ventricular septum. This heart is considered an example of Spitzer's type 1 of transposition. In a second case, that of a man aged 58, both the aorta and the pulmonary artery arose from the right ventricle, the ventricular septum being defective. This is Spitzer's type 2 of transposition.

O. T. SCHULTZ.

RACEMOSE ANGIOMA OF BRAIN WITH VARIX FORMATION AND RUPTURE. A. RÜHL, Beitr. z. path. Anat. u. z. allg. Path. **82**:163, 1929.

A man, aged 32, became suddenly ill with symptoms of cerebral irritation: nausea, vomiting, opisthotonos, stiffness of the neck, a positive Kernig sign and loss of consciousness. Consciousness returned the next day but the patient

remained in a semi-irrational condition. The temperature was normal. On admission to the hospital on the eighth day, papilledema and multiple retinal hemorrhages of the left eye were noted. The cerebrospinal fluid was under greatly increased pressure and was yellowish. Death occurred on the sixteenth day. At necropsy clotted blood was present at the base of the brain and between the cerebellum and the occipital lobes. The lateral ventricles were dilated by partly clotted blood. In the region of the left side of the brain stem and causing an elevation of the floor of the left lateral ventricle was a hemorrhagic tumor mass the size of a hen's egg. Macroscopically, two varices the size of a cherry could be detected. Rupture of both of these had occurred. Microscopically, the mass was a venous racemose angioma. Previously reported similar cases, which are few in number are briefly reviewed.

O. T. SCHULTZ.

ATRESIA OF THE AORTA IN AN ADULT. K. DECKNER, Beitr. z. path. Anat. u. z. allg. Path. **82**:172, 1929.

In a woman, aged 70, the aorta was found obliterated at the level of attachment of the obliterated ductus arteriosus. An adequate collateral circulation had been formed by way of the deep cervical, lateral thoracic, internal mammary, intercostal and inferior epigastric arteries. Terminal thrombosis had occurred in some of these collateral vessels and in the aorta below the point of atresia. Atresia of the aorta at the level of the ductus arteriosus is not a rare anomaly in the new-born, but is usually not compatible with a long duration of life. The author could find twenty examples of the lesion in the literature. He expresses his belief that the effects of the lesion are slight if the obliteration of the ductus and of the aorta occur so gradually that there is time for the development of a collateral circulation.

O. T. SCHULTZ.

ANTHRACOTIC CAVITY FORMATION IN THE LUNG. G. STERN, Beitr. z. path. Anat. u. z. allg. Path. **82**:178, 1929.

The older literature contains many references to cavity formation in the lung which was considered to be the result of anthracosks. In England the condition was known as black phthisis, in Germany as phthisis atra, and in France as phthisie charboneuse. More exact knowledge of the tuberculous process established that most of these cases were examples of cavity formation due to tuberculosis. Anthracosis came to be looked on as a relatively harmless process, although silicosis was known to cause marked changes in the lung. In the later literature, instances of nontuberculous cavity formation in anthracotic lungs are few. The author presents such a case. The formation of the multiple cavities noted is ascribed to degeneration, necrosis and softening of chronically inflamed tissue, the vascular supply of which becomes inadequate for proper nutrition of the tissue. The following percentages of some of the constituents are of interest: normal lung: dried substance, 16 per cent, ash 4.5 per cent of dried substance coal dust 0.6 per cent, silicate dust 0.2 per cent; anthracotic lung with cavity formation: dried substance . . . 20 per cent, ash 8.5 per cent of dried substance, coal dust 12.6 per cent, silicate dust 1.6 per cent. In view of the comparative inocuousness of coal dust in the lung, the author believes that the increased silicate content is an important factor in determining the formation of cavities.

O. T. SCHULTZ.

MALDEVELOPMENTS OF THE LUNG. F. ALTMANN, Beitr. z. path. Anat. u. z. allg. Path. **82**:199, 1929.

On the basis of thirteen examples, most of them in new-born infants or stillborn fetuses, Altmann makes a classification of maldevelopments of the lung into which he thinks most, if not all of the previously described examples can be fitted. The maldevelopments are divided into two main groups: those characterized by disturbances of growth, and those characterized by failure of develop-

ment of the epithelial or mesenchymal element in a circumscribed area with overgrowth of the other element. Cyst formation may occur in either group. The first group is subdivided into two groups. In one there is simple overgrowth of the bronchi and their end branches. This process may involve a restricted area or a whole lobe or lung. The bronchial overgrowth may be associated with varying degrees of incomplete differentiation. In the second subgroup there is localized overgrowth of the bronchial branches, with a failure of development of the terminal outgrowths of the bronchi. In the second main group the process is one of hyperplastic or blastomatous overgrowth in a localized area of elements normally present, but because of the proliferation of one or the other element, the relationship of the tissue elements to each other is an abnormal one. The second main group is subdivided into two subgroups, depending on whether the epithelial or mesenchymal elements are hyperplastic. Two examples of mediastinal cyst are included; these the author is also able to place in his classification.

O. T. SCHULTZ.

HISTOLOGY OF CEPHALHEMATOMA. O. HATSCHEK, Beitr. z. path. Anat. u. z. allg. Path. **82**:268, 1929.

Hatschek says that the histology of the reparative changes in cephalhematoma has not been previously described. He presents in detail a microscopic study of the process based on two examples. The first came from a child which lived seventeen days, the second from one which lived three months. In each case there was congenital internal hydrocephalus. In the first case there was an external and an internal hematoma; in the second case, only an internal one. In the first case, the bone between the central portions of the two hematomas was necrotic. Organization of the hematoma occurs at the periphery by the formation of cellular connective tissue. The pericranium or the dura, lifted off by the hemorrhage, acts as a cambium layer and forms new bone in the organization tissue. Toward the central portion, away from the lateral margins, the area of hemorrhage had become encapsulated by a thin layer of dense fibrous tissue. Connective tissue proliferation was no longer active in this tissue, but new bone formation was in process, both above and below the encapsulated hemorrhage. Red blood corpuscles had disappeared from the latter. The author believes that the hematoma must finally be replaced by new formed bone, which forms a layer considerably thicker than the normal skull. Although he has not seen the still later stages, he believes that the thick layer of new formed bone is gradually reduced to normal thickness by pressure exerted on its inner surface by the growing brain. He appends a description of the proliferative changes and new bone formation in a syphilitic cranial osteophyte into which hemorrhage had occurred at birth.

O. T. SCHULTZ.

APLASIA OF THE THYROID IN RELATION TO ENDOCRINE FUNCTION DURING FETAL LIFE. E. J. KRAUS, Beitr. z. path. Anat. u. z. allg. Path. **82**:291, 1929.

In his discussion of the question whether the endocrine organs manifest their specific functions during fetal life, Kraus quotes the opinion of E. J. Thomas that these organs exhibit no activity during the fetal period under normal conditions, but that the fetus is dependent for its endocrine hormones on those of the maternal circulation. As a contribution to this question, he presents a study of a male infant 47 cm. long, which died on the day of birth. In addition to multiple anatomic maldevelopments, the following changes were noted in the endocrine system: complete aplasia of the thyroid, hypoplasia of the thymus, hyperplasia of the hypophysis and suprarenals. The parathyroids, the interstitial cells of the testes, and the islands of Langerhans were normal. The hypoplastic thymus revealed microscopic evidence of involution. The hyperplasia of the hypophysis involved the anterior lobe and was due to an increase in the number of large chromophobe cells. Both the cortex and medulla of the suprarenals

were hyperplastic and the medulla gave the chrome reaction, which is accepted as evidence of epinephrine formation. Kraus interprets the changes noted in the endocrine system as proof that a mutual inter-relationship of the organs of this system exists during fetal life and that the endocrine system functions specifically before birth.

O. T. SCHULTZ.

THE MARROW OF THE HUMAN FEMUR. W. HALLERMANN, Beitr. z. path. Anat. u. z. allg. Path. **82**:345, 1929.

Although much attention has been paid to changes in the marrow which manifest themselves in alterations of the cellular composition of the circulating blood, little attention has been given, according to Hallermann, to changes in the marrow that do not lead to deviations from the normal blood picture. His report is based on the study of the marrow of the femur of 1,400 necropsies made during a period of about eight months in Schmorl's institute, where the right femur is routinely removed for examination; in many cases the left femur was also removed. A frontal section of the bone permitted examination of the marrow from the head of the condyles. Hallermann's study was directed along three lines: the relative distribution of red and fatty marrow in the femur, localized changes in the femur marrow without alterations in the peripheral blood, and changes peculiar to the fatty marrow alone. Red marrow was found to be present in most of the femurs examined, being usually limited to the upper third of the bone. It occurred as small islands in the fatty marrow, as a narrow zone immediately beneath the cortical layer of the bone, in the neck of the femur as a red streak in the long axis of the bone, or as a layer beneath the cartilage of the head. The localized changes noted were hemorrhages into the marrow, cyst formation, and necrosis. Localized hemorrhages were seen in forty cases, and cysts in fourteen. The lesion to which the author devotes most attention is one limited to the fatty marrow, often visible in the gross as a yellow zone about an older area of hemorrhage. Microscopically, such areas were composed of necrotic fat cells and of new formed cells which contained fat droplets. The necrosis, which was often associated with fibrin formation, is held to be due to poor nutrition of fat cells about areas into which hemorrhage has occurred. The new formed cells containing fat, the author believes to be reticulum cells which have stored the fat liberated by necrotic fat cells. He does not consider them true fat cells or lipoblasts, and their formation is not a process of regeneration of true fatty marrow.

O. T. SCHULTZ.

PRIMARY ARTERIOSCLEROSIS OF THE PULMONARY ARTERY. U. STEINBERG, Beitr. z. path. Anat. u. z. allg. Path. **82**:443, 1929.

In this communication Steinberg, who had discussed the subject of secondary pulmonary arteriosclerosis in the preceding number of the *Beiträge*, reports two cases of the primary form of the condition. The patient in one was a man aged 50; the other was a woman aged 52 years. The man had had an attack of acute rheumatic polyarthritis a year before his death. In each case a clinical diagnosis of valvular disease of the heart had been made. Common to both cases was marked hypertrophy of the right ventricle, and slight macroscopic arteriosclerosis of the larger pulmonary vessels and general circulation. In the first case the smaller arteries and arterioles of the lung were the seat of a marked and widespread productive inflammatory process, which was most marked in the adventitia and had led to great narrowing of the lumen. The process is held to have been infectious in origin and probably rheumatic. In the second case, the small arteries and arterioles were involved in an arteriosclerotic process which was characterized by intimal thickening that greatly decreased the lumen. The hypertrophy of the right ventricle was ascribed in each case to the mechanical hindrance to the pulmonary circulation offered by the narrowing of the arterioles. In each case many of the vessels were thrombosed.

O. T. SCHULTZ.

ACUTE PEPTIC GASTRITIS. E. HAMMER, Beitr. z. path. Anat. u. z. allg. Path. **82**:485, 1929.

As a contribution to the much discussed question as to whether the gastric juice can injure the previously normal mucosa, as Aschoff and his pupils have maintained, or can exert its digesting action on the mucosa only after the latter has been already damaged, as most others have contended, Hammer presents a study of a process which he terms acute peptic gastritis. To obtain material for study, stomachs removed at necropsy done immediately after death were fixed at once and subjected to serial microscopic examination. In four stomachs with acute macroscopic ulcers in the pyloric region and in one with a similar lesion in the duodenum, the mucosa contained multiple microscopic lesions which are described in detail. These were usually situated in the deepest parts of the furrows, and in all but one case they were limited to the pyloric region. The lesions, which rarely penetrated deeper than the upper third of the mucosa, consisted of a superficial zone of fibrinoid hyaline necrosis. At the margin this zone was covered by the surface epithelium; at the center the latter was absent. Some of the capillaries within the zone of necrosis contained hyaline thrombi. About the area of necrosis was a zone of leukocytic infiltration, in which fibrin was sometimes present. The capillaries in this zone were not thrombosed, but contained an increased number of leukocytes. The process is interpreted as an inflammatory reaction in a previously undamaged mucosa, the reaction being brought about by the caustic action of the gastric juice on the living tissue. The explanation of the focal character of the lesions offers difficulties. Hammer suggests that during contractions of the stomach, the gastric juice may be held for longer periods within the furrows of the mucosa. Other factors may have a part in rendering the mucosa subject to the action of the gastric juice, but the author contends that the acute inflammatory reaction is the result of the direct and primary action of the gastric juice on the tissue.

O. T. SCHULTZ.

ATHEROSCLEROSIS OF THE CORONARY ARTERIES OF THE HEART. KAPITOLINE WOLKOFF, Beitr. z. path. Anat. u. z. allg. Path. **82**:555, 1929.

Under arteriosclerosis are included a number of processes, one of which is atherosclerosis. By atherosclerosis the author understands a condition characterized by lipoid infiltration of the deepest layer of the intima, by fibrous thickening of the intima over the area of infiltration, and by the formation of nodular plaques that contain lipoids, chiefly cholesterol or its esters, and that protrude into the lumen of the vessel. Previous studies of atherosclerosis of the coronary arteries have been chiefly statistical in character. The author made a systematic macroscopic and microscopic study of the coronary arteries of 120 persons ranging in age from 4 months to 75 years, with a fairly even distribution among the different decades. There were fifteen cases in each of the first six decades except the first, in which there were twenty, and the fifth and sixth, in each of which there were sixteen; there were fourteen cases in which the patients were more than 60 years of age. The normal functional and histologic characteristics of the coronary arteries differ from those of any other part of the arterial system. The coronary circuit is a short one and is the first one to be given off from the aorta; hence it is subjected to a higher intra-arterial pressure than exists elsewhere. This functional characteristic manifests itself by a physiologic increase of elastic tissue in the intima, to such a degree that by the second or third decade the intima is as thick as the media, a normal structure exhibited by no other artery. Atherosclerotic changes begin to make their appearance in the coronary arteries during the first decade of life and increase in frequency with each succeeding decade. In the series studied they were noted in 2 of 20 cases of the first decade, in 9 of 15 cases of the second decade, in 12 of 15 cases

of the third decade, in 13 of 15 cases of the fourth decade and in every case of all later age periods. In the earlier periods of life they occurred in the main trunks of the coronary arteries and consisted only of flecklike areas of lipoid infiltration. With increasing age, the process progressively involved the rest of the coronary system, the left coronary almost without exception being involved to a greater degree than the right. At about the middle of the second decade, fibrous thickening of the intima over the areas of infiltration makes its appearance and becomes progressively more marked at later ages. Nodular atherosclerotic plaques begin to appear during the fourth decade; these in their turn become progressively more numerous as the age increases. The progressive increase in the incidence of atherosclerotic changes, and the appearance of fibrous thickenings and of nodular plaques as the age increases indicate, as does also the histology of the lesions that the three types of lesions, that is, pure lipoid infiltration, thickening of the intima, and the formation of atherosclerotic plaques, are progressive stages of a single process. The primary condition in this process is lipoid infiltration, as postulated by Anitschkow, in whose laboratory the work was done. Thickening of the intima and plaque formation are secondary reactions to the infiltration. The plaques as a rule contained much lipoid material. In a few instances, the plaques contained little or no lipoid. In such lesions there was noted an ingrowth of capillaries into the plaque from the media. The capillaries were surrounded by xanthoma cells. Resorption of lipoid material from the plaques may therefore occur. The regressive changes seen in the plaques were crystallinization of cholesterol, atheromatous softening and calcification. The most important general factor in atherosclerosis is the abnormality of metabolism which leads to lipoid infiltration. A second general factor is the increased arterial pressure. Local factors which influence the frequency and distribution of the process in the coronary arteries are the relatively higher pressure in the coronary circuit and the structure of the coronary arteries.

O. T. SCHULTZ.

CHOLESTEROL PERICARDITIS. H. HERZENBERG and I. FAFIUS-GORDON, Centralbl. f. allg. Path. u. path. Anat. **46**:97, 1929.

Autopsy of the body of a 21 year old woman disclosed a scirrhous carcinoma of the stomach and a bronchiogenic carcinoma of the left upper lobe with extensive metastases. The left pleural cavity contained about 250 cc. and the pericardial sac about 750 cc. of a yellow fluid. The epicardium contained metastases and in addition warty yellow growths and granulations. Presumably the authors encountered a case of Hydrops adiposus seu chyliformis, because analyses of the pericardial fluid yielded 7 per cent albumin and 5.5 per cent cholesterol. The carcinoma metastases had invaded all layers of the epicardium and blocked the lymphatic drainage.

GEORGE RUKSTINAT.

SCLEROSIS OF MULTIPLE ENDOCRINE GLANDS. P. F. RICHTER, Deutsche med. Wchnschr. **55**:1760, 1929.

In a woman, aged 62, in whom axillary and pubic hair and eyebrows had fallen out thirty years before, there was noted increasing muscular weakness, slow scanning speech and apathy for two years. Shortly before death there were alternating periods of unconsciousness and consciousness. The essential clinical signs were a marked secondary anemia, a systolic blood pressure of 85 and a basal metabolic rate of less than normal. The postmortem examination disclosed a marked atrophy of the hypophysis, thyroid gland, suprarenal glands and ovaries. Histologically there were marked fibrous changes and atrophy of these tissues. It was thought that a puerperal infection thirty years previously might have initiated these changes.

PAUL J. BRESLICH.

ARTERIAL CHANGES IN POISONING WITH IRRADIATED ERGOSTEROL (VIOSTEROL). R. HÜCKEL and H. WENZEL, Ztschr. f. Kreislaufforsch. **21**:409, 1929.

Viosterol was given to rabbits by mouth in amounts of from 9 to 700 mg. within from one to three weeks, in single doses of from 1 to 100 mg. The animals lost weight and nearly all of them died spontaneously. Macroscopic examination of the arteries of rabbits which had not received more than a total dose of 20 mg. revealed elevations in the beginning of the aorta. In animals which had received from 50 to 70 mg. of viosterol the ascending portion of the aorta was thickened and its inner surface was rough and contained hard plaques that projected into its lumen. In the descending portion of the aorta the changes were less marked but at the transition from the thoracic aorta to the abdominal aorta they were pronounced; the lower lumbar portion of the aorta was macroscopically free from changes. In animals which had received from 100 to 200 mg. of viosterol the process involved the entire aorta. In all the animals which had received still larger doses the whole aorta had been converted into a rigid tube which curled outward when incised longitudinally. The extensive, hard deposits of calcium with intervening large and small aneurysms and the rough inner surface of the aorta constituted a characteristic picture of the vascular injuries produced by viosterol. Compared with these changes in the aorta, the changes in the large arteries were unimportant. Microscopically, calcification could not be demonstrated in animals that had received a total dose of only 12 mg.; in these animals, however, marked changes in the media were noted. The outer layers of the media were loosened and presented a honeycomb marking. Large vesicular cells which did not contain fat were present. The irregularity of the inner surface of the aorta was particularly marked in one animal which had received a total of 14 mg. In the projections the fibers of the media were loosened. Particularly the inner layers of the media were characterized by localized areas that stained poorly; in these areas the elastic fibers were decreased in number and showed interruptions in their continuity. In some places in the aorta structures resembling giant cells were noted, together with degeneration of the nuclei and fibers and calcification. In areas in which the calcification of the media was marked, the overlying intima had become necrosed whereas the outer layers of the media were almost unchanged. From this study it is evident that the first morphologic changes in the development of the process occur in the media. Examination of the arteries of the kidney revealed that here, also, the pathologic process began in the media. Changes in the media of the arteries of the kidney were noted in animals that had received only 9 mg. of viosterol. Contrary to the changes in the aorta, large doses of viosterol produced extensive primary necrosis of the media of the arterioles of the kidney. The author emphasizes the fact that only minute quantities of viosterol were required to produce marked injury of the wall of the aorta and of the arteries of the kidney.

THE INFLUENCE OF INFECTION ON THE THYROID GLAND AND ITS SIGNIFICANCE IN THE ETIOLOGY OF GOITER IN HOLLAND. H. L. KOESTER, Dissertation, Utrecht, 1929.

In Holland, the normal thyroid gland shows a different histologic structure according to sex, age, locality, menstruation and pregnancy (43 autopsies). The normal weight of the organ varies between 20 and 50 Gm., the average weight being 30 Gm. in the adult. After the age of 50, involution of the thyroid begins. The difference between normal and goitrous thyroid gland applies to the form and size of the acini, amount and quality of the colloid and height and proliferation of the epithelium. The influence of different acute and chronic infectious diseases on the thyroid gland was studied in ninety-five autopsies. Following acute infectious diseases, typhoid fever and septicemia, the thyroid gland showed hyperemia, decrease of colloid and desquamation of the epithelial cell layer. These changes did not resemble the histologic appearances in goiter.

Bacteriologic examinations of twenty-seven goiters and of the feces of twenty goitrous patients did not support the theory that goiter is due to some local or

intestinal infection. In the feces of goitrous persons, no other micro-organisms and no more parasite eggs were found than in goiter-free people.

In extensive experiments on rats, broth cultures of feces from patients with goiter were fed for four months, but the animals did not develop hyperplastic changes of the thyroid gland.

C. A. HELLWIG.

Microbiology and Parasitology

THE RELATION OF MOISTURE TO THE DISTRIBUTION OF HUMAN TRICHURIS AND ASCARIS. L. A. SPINDLER, Am. J. Hyg. **10**:476, 1929.

The incidence of *Trichuris* in southwestern Virginia was found on the whole to be much lower than that of *Ascaris*. However, in an area differing from those with a low incidence of *Trichuris* only in the amount of moisture in the soil (due to the abundance of shade) the incidence of *Trichuris* was not only higher (61 per cent) than that of *Ascaris* (56 per cent) but the infestations were also quite heavy.

From all the information available (laboratory experiments and incidence data), it was concluded that an important factor in the differences found in the incidence of *Ascaris* and *Trichuris* in regions where habits of soil pollution seemed favorable for their spread was a difference in the moisture requirements of the eggs. This was indicated by the fact that wherever the soil was kept moist on account of high rainfall or dense vegetation, the incidence of *Trichuris* was, as a rule, high, while a high incidence of *Ascaris* was found in much drier situations.

JOHN PHAIR.

A RAT-FLEA SURVEY OF PEIPING. MARSHALL HERTIG AND TSEFANG F. HUANG. Am. J. Hyg. **10**:521, 1929.

In a rat-flea survey of Peiping extending throughout one year, 6,286 rats were examined. *Xenopsylla cheopis* (98 per cent) and *Ceratophyllus anisus* (2 per cent) were the only rat-fleas found. The average number of *Xenopsylla* per rat was 1.33 for the whole year with a maximum of 3.1 in August and a minimum of 0.3 in February.

Peiping's freedom from bubonic plague is not to be explained by the absence of the vector, *Xenopsylla cheopis*, but would seem to lie partly in its geographical position and partly in the prevalence throughout most of the year of climatic conditions, notably the extreme dryness and the cold winter, unfavorable to the establishment and maintenance of plague. (Brooks, 1917.)

JOHN PHAIR.

THE LIFE HISTORIES OF DIPHYLLOBOTHRIUM DECIPIENS AND DIPHYLLOBOTHRIUM ERINACEI. H. C. LI, Am. J. Hyg. **10**:527, 1929.

These life histories have been studied and are presented thoroughly.

JOHN PHAIR.

ON THE VIRUS OF RAT ANEMIA AND ITS TRANSMISSION BY THE RAT LOUSE, POLYPLAX SPINULOSA. CALISTA P. ELIOT AND W. W. FORD, Am. J. Hyg. **10**:635, 1929.

The splenectomized Wistar rat was used for the study of insect transmission of *Bartonella muris*. Living lice were picked from infected stock and placed on these test animals. Seven of nine rats died of anemia within from three to five days after the first appearance of *Bartonella* and eighteen to twenty-eight days after infestation with the lice. One rat recovered from the first attack and died in a relapse on the thirty-third day after infestation. One rat showed a prolonged recovery. One attack of *Bartonella* anemia does not protect the splenectomized

rat against subsequent spontaneous or induced attacks, while in the adult normal rat with intact spleen the disease cannot be produced. The blood of sixty-two wild rats has been examined for *Bartonella muris*. Of these, fourteen showed a definite infection. Many of the wild rats examined harbored trypanosomes and leptospiras. How much these other infections contribute to the breaking down of the defense mechanism of the spleen and so to the appearance of *Bartonella muris* has not been determined.

JOHN PHAIR.

CHEMICAL CHANGES IN THE GROWTH OF AVIAN TUBERCLE BACILLI ON LONG'S SYNTHETIC MEDIUM. A. G. RENFREW, S. L. BASS and T. B. JOHNSON, Am. Rev. Tuberc. **20**:114, 1929.

The growth of avian tubercle bacilli was followed analytically for a period of sixteen weeks, using the methods previously described (*Am. Rev. Tuberc.* **18**:508, 1928) for the study of human tubercle bacilli. The most striking fact revealed was the difference in the production of the substances of carbohydrate nature. The copper-reduction curve suggests that these two micro-organisms do not produce the same carbohydrate combination in the metabolism.

H. J. CORPER.

CHEMICAL CHANGES FROM THE GROWTH OF TIMOTHY BACILLI ON LONG'S SYNTHETIC MEDIUM. SHAILER L. BASS and TREAT B. JOHNSON, Am. Rev. Tuberc. **20**:122, 1929.

The growth of timothy bacilli was followed analytically for a period of sixteen weeks, using the methods previously described for the study of human and avian tubercle bacilli. The most striking difference between timothy bacilli and human tubercle bacilli is revealed by the analyses for reducing sugars. Timothy and avian bacilli are both characterized by the formation of sugar of low reducing powers, while the reducing power of the sugar formed in the case of human tubercle bacilli is variable and increases to a maximum. The results of analyses indicate that the same sugar combination is not functioning in all three bacterial organisms.

H. J. CORPER.

A DEVELOPMENTAL CYCLE OF THE TUBERCLE BACILLUS AS REVEALED BY SINGLE-CELL STUDIES. MORTON C. KAHN, Am. Rev. Tuberc. **20**:150, 1929.

With the aid of Chamber's micromanipulator, it was possible to grow single tubercle bacilli or small groups of from two to six organisms in individual droplets of Long's medium for varying periods of time. Under the experimental conditions described, the H37 strain of human tubercle bacilli did not multiply solely by simple fission but underwent a more complex type of reproduction consisting of: (*a*) the initial segmenting of the rod into three or more ovoid units; (*b*) the division of these units into diplococcoid forms; (*c*) subsequent grouping and reduction of these elements to a mass of dust fine particles, from which extremely small and delicate rods were found to sprout, and (*d*) the later development of these tiny rods into the mature tubercle bacillus. A so-called "wild" strain of tubercle bacillus from urine was found to undergo an identical developmental process. Some of the stages of growth were found to be nonacid-fast. The possible relation of the forms encountered to those previously described by other observers is discussed.

H. J. CORPER.

THE TISSUE REACTIONS TO PRIMARY INFECTION AND REINFECTION WITH THE TUBERCLE BACILLUS. LEROY U. GARDNER, Am. Rev. Tuberc. **20**:201, 1929.

Histologic study serves as a qualitative basis for comparing the effects of primary infection and reinfection with the tubercle bacillus in the peritoneal

cavity. It demonstrates that the subperitoneal tissues are not exceptional in their behavior, and that these tissues also meet primary infection with a slow proliferation, and reinfection with an inflammation accompanied by accelerated proliferation. Both sensitized and nonsensitized animals respond immediately to the injection of tubercle bacilli with an acute inflammation. But in the primary infection, this reaction is nonspecific, and it completely disappears after forty-eight hours. In the reinfection, the nonspecific exudation is accentuated by hypersensitiveness, and inflammatory phenomena do not disappear, but continue with increasing severity throughout the course of the disease.

H. J. Corper.

Culturing Bovine Tubercle Bacilli. Max Evanoff and H. C. Sweany, Am. Rev. Tuberc. **20**:227, 1929.

A direct method is described for obtaining growths and identifying tubercle bacilli from bovine material. The bovine bacilli isolated grow directly on a cream-egg-milk medium, but not on a glycerol-egg-milk medium or other glycerol-egg medium. There appears to be a shower of organisms associated in the lesions of cattle that resemble paratubercle bacilli, some of which appear to be tuberculogenic after animal passage. Direct inoculation and treatment with 3 per cent hydrochloric acid and 3 per cent sodium hydroxide are recommended for the best results.

H. J. Corper.

The Effect of Ultraviolet Light on Corneal Tuberculosis in Rabbits. H. F. Newton, Arch. Surg. **18**:1542, 1929.

When tubercle bacilli are injected into the cornea and it is then exposed to ultraviolet irradiations before it shows a gross tuberculous lesion, the bacilli are destroyed and corneal tuberculosis is prevented. On the other hand, if the lesion has developed, ultraviolet irradiation does not sterilize the cornea, and even accelerates the ulceration and exudate.

N. Enzer.

The Agent of Herpes. Hans Zinsser and Fei-Fang Tang, J. Immunol. **17**: 343, 1929.

The virus deteriorates rapidly in the presence of free oxygen, but survives for considerably longer periods under anaerobic conditions. The presence of serum also favors survival of the virus. The serum of convalescents from encephalitis may neutralize herpes virus; this action of serum is independent of complement. Efforts to immunize with the dead herpes agent failed entirely.

Observations Following the Feeding of Salmonella Enteritidis to Rats on Diets Varying in Vitamin A Content. Elizabeth Verder, J. Prev. Med. **3**:489, 1929.

Two of nine rats on a diet containing an adequate amount of vitamin A developed a profuse watery diarrhea from twelve to twenty-four hours after swallowing a suspension of living *S. enteridis,* while none of the ten rats on diets containing very small amounts of this vitamin showed any symptoms of "food-poisoning." In this series of experiments neither the ability of this strain of *S. enteritidis* to invade the tissue nor its capacity to persist and probably increase in numbers in the duodenum were of importance in provoking "food-poisoning" symptoms. The cultures of the spleen, livers, lungs, kidneys and heart blood of the rats on deficient diets showed that most of them were harboring organisms commonly found in the intestinal flora, while the organs of the rats on adequate diets proved sterile. *S. enteritidis* was isolated from the spleen, livers, lungs and kidneys, as well as from the duodenum, of many of the deficient-diet to which this organism has been fed.

Author's Summary.

BOTULISM RESULTING FROM CONSUMPTION OF CANNED ONIONS. STEWART A. KOSER and DOROTHY O. REITER, J. Prev. Med. **3**:499, 1929.

An outbreak of botulism involving two cases with one death in Chicago in 1929 was caused by Italian canned onions (shallots). Botulinum toxin, type B, was demonstrated in the blood serum but not in the spinal fluid in the fatal case. The presence of *Clostridium botulinum*, type B, was also demonstrated in the small intestine (ileum). Inspection of approximately 400 cans of the same shipment revealed slightly over 1 per cent of swelled cans. One swelled can, secured from a retail store, was found to be toxic and a culture of *Clostridium botulinum*, type B, was isolated from this.

AUTHORS' SUMMARY.

QUAIL AS A POSSIBLE SOURCE OF TULARAEMIA INFECTION IN MAN. R. R. PARKER, Pub. Health Rep. **44**:999, 1929.

Quail should be considered at least potential agents of human infection with tularemia.

AUTHOR'S SUMMARY.

THE NATURE OF THE LESIONS IN GENERALIZED VACCINIA IN RABBITS. JAMES MCINTOSH and R. W. SCARFF, J. Path. & Bact. **32**:551, 1929.

With certain strains of virus, generalized vaccinia in rabbits regularly occurs, both after scarification and after intravenous inoculation. All the lesions of generalized vaccinia show the histologic picture of an infective granuloma affecting primarily the smaller blood vessels with endothelial proliferation, necrosis of tissues and subsequent fibrosis. The visceral lesions are due to the direct action of the vaccinia virus and not to the activation of a latent infection.

AUTHORS' SUMMARY.

THE EXOTOXINS OF STAPHYLOCOCCUS PYOGENES AUREUS. F. M. BURNET, J. Path. & Bact. **32**:717, 1929.

The agent obtained in staphylococcal culture filtrates that is capable of killing rabbits acutely on intravenous injection is a true exotoxin. The three activities of such filtrates in causing hemolysis in vitro necrotic skin lesions on intradermal injection into rabbits and acute death of rabbits after intravenous injections are manifestations of a single antigenic substance. A typical anatoxin can be obtained with full antigenic power but no toxicity and diminished hemolytic power. The toxin is produced in vivo in rabbits infected with a toxigenic strain of staphylococci. If rabbits are actively or passively immunized to the toxin, their survival time after intravenous injection of virulent staphylococci is increased. The bearing of these observations on the pathology of human staphylococcal infections and more particularly on the fatalities at Bundaberg, Queensland, is briefly discussed.

AUTHOR'S SUMMARY.

EXPERIMENTS TO DETERMINE WHETHER THERE IS A FILTERABLE FORM OF THE TUBERCLE BACILLUS. S. R. GLOYNE, R. E. GLOVER and A. S. GRIFFITH, J. Path. & Bact. **32**:775, 1929.

None of the animals inoculated with filtrates showed any of the obvious signs of tuberculosis, such as enlargement of the lymphatic glands with fibrosis or caseation. Tubercle bacilli were found in only one microscopic preparation; these were traced to an imperfectly cleansed scalpel which though it had been boiled for twenty minutes in strong soda solution showed traces of coagulated pus at the tip. The eight guinea-pigs inoculated with apparently slightly enlarged glands or organs containing doubtful foci were free from any sign of tuberculosis when killed from 58 to 115 days later, average 88 days. All the young guinea-pigs were perfectly healthy. Our experiments have failed to demonstrate the existence of a filtrable form of the tubercle bacillus.

AUTHORS' SUMMARY.

TYPES OF TUBERCLE BACILLI IN HUMAN TUBERCULOSIS. A. STANLEY GRIFFITH, J. Path. & Bact. **32**:813, 1929.

The observations and experimental investigations recorded here show that tuberculous lesions, whether produced by human or by bovine tubercle bacilli, and wherever situated, may undergo spontaneous arrest and cure.

AUTHOR'S SUMMARY.

THE BACTERIAL CONTENT OF THE NORMAL CORNEA. ADOLF BLANK, Arch. f. Ophth. **122**:661, 1929.

A study of the germ content of the normal cornea with relation to that of the contiguous normal conjunctiva was made. With careful technic smears were obtained from the eyes of 100 normal persons and cultures were made on blood serum. In the same manner the bulbar conjunctiva also was investigated. The cultures were examined after from twelve to twenty-four hours. (Observations for pneumococcus must be made early, for these are easily overrun by the rapidly growing staphylococcus and *B. xerosis.*)

Results were often different in the two eyes of the same subject. In only 35 per cent were results for both eyes alike. In over half of the eyes examined, the cornea and conjunctiva were alike; with few exceptions the bacteria from the cornea grew more sparsely in the culture than those from the conjunctiva.

In only seven eyes were pneumococci found on the cornea and not on the conjunctiva; hence the author could not verify Lindner's statement that pneumococci are found on the cornea alone in the greater percentage of cases. Total results were as follows: pneumococci were found in 52 per cent of the aggregate eyes examined; staphylococci, in 78 per cent; *B. xerosis,* in 66.5 per cent; diplobacilli, in 4 per cent; and *B. subtilis,* in 2.5 per cent. Pneumococci were especially frequent in the eyes of older persons and slightly more frequent in men than in women. In general, it must be admitted that along with staphylococci and *B. xerosis,* chain diplococci (probably for the most part belonging to the pneumococcus group) grow on the conjunctiva and frequently, perhaps always, on the normal cornea also.

CHARLES WEISS.

IS A BACTERICIDAL CHEMOTHERAPY OF TUBERCULOSIS POSSIBLE? F. JESSEN, Beitr. z. Klin. d. Tuberk. **72**:184, 1929.

Attempts to stain tubercle bacilli vitally in diseased rabbits failed. After repeated injections of fuchsin, some of the dye was demonstrable in the center of small necroses.

MAX PINNER.

PATHOLOGY OF HUMAN TUBERCULOSIS. P. SCHÜRMANN, Beitr. z. path. Anat. u. z. allg. Path. **81**:568, 1929.

The author presents a minute and detailed study of human tuberculosis, undertaken with the object of testing the correctness of Ranke's hypothesis that human tuberculosis always runs a cyclic course. This course is composed of three stages: a first stage of primary localization of the infection, with local growth by direct continuity, spread to the contiguous lymph nodes, and relative local allergic tissue hypersusceptibility, manifested by a proliferative reaction about the tubercles; a second stage of generalization, due to direct, lymphogenous, hematogenous and intracanalicular spread of the process, with generalized allergic tissue hypersusceptibility, manifested by an exudative inflammatory reaction about the tuberculous foci; and a third stage of relative humoral immunity, in which the lesions increase by direct growth. For the purpose of his study Schürmann examined carefully for tuberculosis 1,000 consecutive unselected bodies which came to

necropsy in Fahr's institute at Hamburg. Infants under 2 months of age were not included. The lungs were removed in toto, fixed by intratracheal or intravascular injection, studied roentgenographically, sectioned frontally for gross and photographic study, and the necessary microscopic preparations made. This communication gives the observations in the cases in which the tuberculous process was generalized; there were fifty-seven of these cases in the series. In two cases the primary focus could not be detected. In forty-six the primary focus was single, and in nine multiple. In 78 per cent of the forty-six cases with a single primary focus, the latter was situated in the lung; in the intestine in 17 per cent, and in the neck and the middle ear each one. The anatomy of the process with reference to the various modes of spread is fully described and illustrated. The author speaks of his observations as the anatomic substrate of the generalization of tuberculosis. He presents no conclusions as to the correctness of the Ranke hypothesis, reserving this for a later communication, in which the cases without generalization will be discussed.

O. T. Schultz.

Etiology of Lymphogranulomatosis. Natalia Busni, Virchows Arch. f. path. Anat. **268**:614, 1928.

From the blood and organs of the patient with lymphogranuloma one and the same micro-organism is constantly obtained after inoculation of suitable culture mediums. The presence of micro-organisms in the blood (in contrast to Grumbach's idea) shows no relationship with the rise in temperature. As regards its morphologic properties, the micro-organism obtained is similar to the tubercle bacillus. The micro-organism appears as an acid-fast bacillus only in a definite stage of its development. It is possible that several observers who described different types of the causative organism have observed in reality one and the same micro-organism but under different conditions of its development and nutrition. The introduction of large masses of these micro-organisms gives rise in the guinea-pig to a disease corresponding to the lymphogranulomatosis in man. The presence of these micro-organisms in all the tissues of the patient makes one believe that lymphogranulomatosis is a generalized infectious disease. On inoculation of guinea-pigs with cultures of organisms of lymphogranulomatosis and of tuberculosis, the structure of the two different granulomas is maintained. Lymphogranulomatosis is, after all conjectures, an independent disease having no connection with tuberculosis. There are: (*a*) the difference in the course and outcome of these two diseases in man; (*b*) the difference in behavior of the guinea-pigs to these diseases; (*c*) the constant presence of micro-organisms in the blood of the lymphogranulomatous patient, and finally, the most important (*d*) the difference in the histologic structure of both granulomas.

Author's Summary.

The Rôle of Mixed Infection in Tuberculosis. J. R. F. Rassers, Ztschr. f. Tuberk. **54**:198, 1929.

The tubercle bacillus requires for its growth nitrogenous protein-split products, preferably amino-acids and ammonia; it seems to be unable to utilize higher compounds, such as peptones, unless free amino-acids are present. Chemical analysis of tuberculous caseous material showed that it did not contain any free amino-acids. The same was true of sterile pus, experimentally produced by injections of turpentine oil into a dog. Any sample of pus that contained streptococci or staphylococci contained free amino-acids. It is well known that caseated tubercles contain but few tubercle bacilli, while (always secondarily infected) cavities contain large numbers. It is probable that the common pyogenic organisms, by splitting proteins to free amino-acids, improve the medium for the growth of tubercle bacilli.

Max Pinner.

Immunology

THE REPRODUCTION OF TUBERCULIN HYPERSENSITIVENESS IN GUINEA PIGS WITH VARIOUS PROTEIN SUBSTANCES. L. DIENES and E. W. SCHOENHEIT, Am. Rev. Tuberc. **20**:92, 1929.

The conditions under which the sensitiveness is produced, the characteristics of the skin reaction, and the result of passive-transfer experiments make it very probable that protein sensitiveness with necrotic skin reactions in tuberculous guinea-pigs must be regarded as a reproduction of tuberculin sensitiveness. The conditions influencing the general tuberculin reaction are so little understood that failure to produce a similar general reaction with egg-white does not weaken the foregoing conclusion. The characteristics of the tuberculin type of sensitiveness (concerning the local reactions) are not dependent on the special properties of the antigens, but are the result of a special type of sensitization which is made possible by the influence of the tuberculous tissue. It is probable that the tuberculin type of sensitiveness is the result of antigen-antibody reaction, as it follows the antigenic specificity, and as the tuberculous tissue has great influence also on antibody production. In the study of immunity in tuberculosis the study of the influence of the lesions on the immunity response of the organism will be of great importance. Certain characteristics of anaphylactic sensitization and production of anaphylactic shock in tuberculous guinea-pigs are also described.

H. J. CORPER.

THE PERMEABILITY OF THE RESPIRATORY TRACT TO ANTIGENS. PAUL F. CLARK, WILLIAM S. MIDDLETON and OTIS M. WILSON, Am. Rev. Tuberc. **20**:106, 1929.

Guinea-pigs may be actively sensitized through respiratory insufflation with a variety of antigens, including one so relatively weak in antigenic properties as old tuberculin. Passive sensitization can be produced by the appropriate use of serum from these actively sensitized animals. Circulating antibodies may be demonstrated by precipitin tests following insufflation with horse serum, a solution of crystalline egg-albumin or a solution of a crystalline protein from the brazil nut. Attempts to give the shocking dose by insufflation after sensitization by the same route produced only minor symptoms of dyspnea and restlessness. In view of the difference in species and the small amount of antigen employed it is believed that these experiments indicate the possibility of the correctness of the hypothesis that the patient in a reported case may have been sensitized by repeated exposures to a tuberculin-laden atmosphere.

H. J. CORPER.

PROTECTIVE IMMUNIZATION WITH B.C.G. (BACILLUS CALMETTE-GUERIN). S. A. PETROFF, Am. Rev. Tuberc. **20**:275, 1929.

Recent studies on the biology of the tubercle bacillus suggest strongly that the micro-organism is not stable and may mutate. The evolution and mutation of all micro-organisms is believed in and therefore the adoption of any method of prophylactic immunization that uses a living micro-organism is opposed. Infants that become infected with a virulent micro-organism and develop a latent tuberculosis may manifest a clinical disease later in life. At present there is not an iota of scientific evidence to show that the same thing may not occur if one infects infants with B.C.G.; there is accredited evidence to suggest that such a catastrophe might happen. The low level of acquired resistance obtained by the implantation of living micro-organisms is all one can expect. Shall one pay the price for such a resistance by infecting the whole race? If one must use a vaccine, it is believed that one composed of dead micro-organisms can accomplish as much as can be obtained with B.C.G.

H. J. CORPER.

THE ANTIGENIC SUBSTANCES OF THE TUBERCLE BACILLUS. L. DIENES, J. Immunol. **17**:85, 157 and 173, 1929.

In the extracts of tubercle bacilli obtained with alcohol of different strength and with methyl alcohol, six different antigens were demonstrable. Three different protein fractions were separated from bacilli and from culture broth; they have different antigenic properties and different isoelectric points. But more than these three antigens are present in protein substances. A protein not precipitable by acids has a strong tuberculin effect and it differs in its antigenic properties from the other proteins. Antibody production is strongest with whole bacilli; antiserums against living bacilli are distinct from those of killed bacilli. The relative amount of antilipoids and antiproteins varies in different batches of antiserums. Both watery and alcoholic extracts stimulate antibody production; the latter antiserums usually contain some antiproteins. A 75 per cent alcohol extract gives rise to two antibodies not present in immune serums prepared by whole bacilli. Certain protein fractions produced precipitins against the specific carbohydrates. Tuberculous guinea-pigs were used for the production of antiserums and for the differentiation of the antigenic properties in vivo. Detailed data on the chemical nature of the various antigens are given. They all contain carbohydrates without apparent connection with the antigenic properties. It seems likely that in the lipoidal substances, protein fractions are responsible for the antibody production. Comparing different protein fractions and their action on guinea-pigs made allergic by various methods, it is evident that different tuberculin preparations have not only quantitative but qualitative differences as well. Only protein substances have a tuberculin effect and all these substances have, with few exceptions, antigenic properties in vitro. The tuberculin effect is probably one of several manifestations of the specific properties of antigens.

MAX PINNER.

ANAPHYLAXIS (RABBIT). L. H. BALLY, J. Immunol. **17**:191, 207 and 223, 1929.

The reactions of the sensitized rabbit on reinjection were studied under ether anesthesia.

THE ANTIGENIC PROPERTIES OF BACTERIOPHAGE. E. W. SCHULTZ, J. S. QUIGLEY and L. T. BULLOCK, J. Immunol. **17**:245, 1929.

The results indicate that the bacteriophage-antibacteriophage reactions closely parallels the toxin-antitoxin reaction, but this does not indicate of necessity that the bacteriophage is a toxin or a ferment.

THE RETICULO-ENDOTHELIAL SYSTEM AND ANTI-BODY PRODUCTION. EDWARD F. ROBERTS, J. Immunol. **17**:273, 1929.

A reticulo-endothelial blockade accelerates by approximately 50 per cent the rate of disappearance of precipitinogen from the circulating blood of the rabbit. Quantitative determinations indicate that the accelerated rate of antigen disappearance is a gradual procedure. Evidence is adduced to show that this effect of a reticulo-endothelial blockade on antigen disposal is attributable to an increase in endothelial permeability rather than to a serum factor.

AUTHOR'S SUMMARY.

STUDIES IN HYPERSENSITIVENESS. ROBERT A. COOKE and W. C. SPAIN, J. Immunol. **17**:295, 1929.

Our studies have not disclosed any fundamental differences between the skin-sensitizing antibodies that are present in the three types of serums studied. Such antibodies seem to be quantatively greater, more active and more regularly found in man than in the animal, for in man, both naturally and artificially sensitized, the clinical evidences of the reaction are largely in the skin and mucous membrane.

Fundamental differences, however, may yet be shown, but as far as facts now go there is no warrant for assuming any difference in the biologic reaction of natural and artificial sensitization in the sense that the reaction is mediated by an antibody mechanism. The varying manifestations would seem to be explained by the fact that the reaction depends on the kind or the relative proportion of the sensitizing antibodies as well as on their number and their activity. But in spite of this recognition of a basic similarity, there is still, clinically, a vast and important difference between the naturally and the artificially sensitive man. This difference lies not in the nature of the reaction but in the constitutional make-up of the man. The naturally sensitive man constitutes a limited group with an inborn capacity for developing a particular, predetermined, and self-perpetuating sensitization. This stamps him as different from his fellow-man, and makes a distinction which is warranted by its clinical value and which for the present should be maintained.

AUTHOR'S SUMMARY.

A NOTE ON THE ANTIGENICITY OF CRYSTALLINE EGG ALBUMIN. R. C. LANCEFIELD and J. H. QUASTEL, J. Path. & Bact. **32**:771, 1929.

Dried, salt-free crystalline egg albumin, recrystallized six times, has been prepared, the analytic results and optical rotation agreeing closely with the figures of Hopkins. Evidence is brought forward to show that no alteration in the properties of the albumin had occurred up to the time of injection into rabbits for the purpose of immunization. Adequate antibody production was observed in all instances after a short course of immunization.

AUTHOR'S SUMMARY.

COMPARATIVE IMMUNIZING EXPERIMENTS ON GUINEA-PIGS WITH B.C.G., SCHRÖDER'S VACCINE, AND KILLED TUBERCLE BACILLI. O. KIRCHNER and H. F. NEWTON, Beitr. z. Klin. d. Tuberk. **72**:97, 1929.

Three series of guinea-pigs of thirty animals each were immunized with B.C.G., Schröder's Vaccine and killed tubercle bacilli, respectively. They, and a fourth series of thirty unprepared guinea-pigs, were infected seven weeks after immunization, intracutaneously, with 0.003 and 0.0003 mg., respectively. The controls died (or were killed) from two to three months after infection. The B.C.G. immunized animals developed the lesions in the regional glands considerably later than the controls, but they all showed extensive tuberculosis at autopsy. The other two methods did not seem to have increased the specific resistance.

MAX PINNER.

THE FATE OF THE TIMOTHY BACILLUS IN THE ANIMAL ORGANISM. W. JELIN, Centralbl. f. Bakteriol. (Abt. 1) **111**:391, 1929; **112**:67, 1929.

Jelin made a histologic study of the organs of twenty-five guinea-pigs, infected with the timothy bacillus. In the lungs, many small nodules developed within from 6 to 14 days, which consisted of two zones, viz., a central zone in which necrobiosis and degeneration of the nuclei of the polymorphonuclear leukocytes was prominent and a peripheral zone composed mainly of epithelioid cells and polyblasts. Later, the surrounding tissue is infiltered by polyblasts, epithelioid cells and fibroblasts. No caseation occurs in the nodules, however. Giant cells are occasionally seen in the nodules. The alveolar walls become thickened, as do those of the blood vessels. Around the bronchi and blood vessels, a rich development of young connective tissue is observed. In the liver, small nodules form around the blood vessels. A deposition of glycogen and fat is observed in the cells of the nodule, but this and the nodules disappear in a few months. A slowly developing glomerulitis and at times some interstitial nephritis are observed in the kidneys. In the spleen and lymph nodes a nonspecific inflammation arises, accompanied by a hyperplasia with, later, a thickening of the capsule.

When dead timothy bacilli are injected, the same processes are observed as described, but they regress more quickly.

PAUL R. CANNON.

THE PHAGOCYTIC POWER OF LEUKOCYTES AS INFLUENCED BY BACTERIOPHAGE. J. C. GERARDS, Centralbl. f. Bakteriol (Abt. 1) **111**:493, 1929.

A virulent strain of Shiga dysentery bacillus and a staphylococcus were used and bacteriophage prepared for each, which were specific. A bacteriophage vaccine was prepared and injected intravenously into experimental animals. The effects of bacteriophage on phagocytosis were tested, and the results indicated that in vitro and in vivo the bacteriophage has a specific opsonizing effect.

PAUL R. CANNON.

EXPERIMENTAL STUDIES ON TISSUE SENSITIVITY OF JOINTS. H. KLINGE, Verhandl. d. deutsch. path. Gesellsch. **24**:13, 1929.

Guinea-pigs were sensitized to a protein by intracutaneous injection, and after varying periods this protein was injected into the knee joints. In some animals the ischiadicus muscles were cut through so as to produce impaired function and ankylosis of the joint. In typical cases a severe arthritis and periarthritis resulted. There was papillary hypertrophy of the synovial membrane, and the joint cavity was enlarged by a cell containing exudate. The character of the exudate was variable. After repeated large doses, there was a phlegmonous or necrotic reaction and a purulent exudate. After smaller doses, there was a mixed leukocyte, monocyte and histiocyte reaction. After many months, there were no leukocytes, although foci of necrosis and subendothelial hyaline degeneration were noted. Bodies similar to Aschoff bodies were encountered in the interstitial tissue of the joint and about the joint vessels. The muscles in the joint region had undergone waxy degeneration. There were glassy nodules on the mitral and aortic valve leaflets and basophilic large celled nodules in the cardiac muscles.

GEORGE RUKSTINAT.

PATHOLOGIC ANATOMY OF TUBERCULOSIS OF LUNG IN MONKEYS. W. H. STEFKO, Virchows Arch. f. path. Anat. **272**:573, 1929.

In *Macacus rhesus, sinicus* and *cynomolgus*, the inflammation in nodules of the lungs is of the anergic type; the connective tissue does not react, and epithelioid cells and giant cells are absent. There is little perifocal reaction; no well defined zones are found. This represents an "alterative" inflammation, a type seldom found in the human being. In cynocephalidae (*Mandrillus pavian*), some epithelioid cells and fibroblasts appear as first signs of allergic inflammation. In one tubercle in the pharynx, epithelioid cells and one giant cell were found. The difference in the mesenchymal reaction between *Macacus, Cynocephalus* and man may be explained by a more primitive character of mesenchyme in man. Man has many primitive characters that monkeys have lost.

ALFRED PLAUT.

PHARMACOLOGY OF RETICULO-ENDOTHELIAL SYSTEM. MARCEL HAENDEL and JUAN MALET, Virchows Arch. f. path. Anat. **273**:127, 1929.

The storage of india ink was decreased by a previous injection of epinephrine. It was increased by hypophysin, insulin, thyroidin and ovarin. Glycerin decreased the storage power; therefore, glycerin extracts of glands cannot be used.

ALFRED PLAUT.

THE PROPERTIES OF DIPHTHERIA TOXIN-ANTITOXIN MIXTURES. H. SCHMIDT and W. SCHOLZ, Ztschr. f. Immunitätsforsch. u. exper. Therap. **58**:98, 1928.

An otherwise fatal diphtheria toxin-antitoxin mixture appears under favorable experimental conditions as nontoxic for guinea-pigs, if the animals are sensitized with the normal serum homologous to the antitoxin. If the guinea-pigs are sensitized to another protein, then no difference is noticed between sensitized and

untreated animals after the injection of the toxin-antitoxin. If the sensitized animals are desensitized through a reinjection of homologous serum, then they are relatively insusceptible to diphtheria toxin. The authors present the supposition that a resulting protein-antiprotein reaction takes place in the body and that certain toxin-susceptible cell groups important for the formation of antitoxin are blockaded. It is suggested that these facts be considered in the practice of active immunization against diphtheria.

ROY C. AVERY.

PRODUCTION OF THROMBOCYTOBARINE. M. N. LEBEDEWA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **58**:255, 1928.

By immunization with bacteria it appears that only spirilla and vibrio possess the capacity to form thrombocytobarine. Bacilli and cocci cannot be employed as thrombocytobarinogen. These thrombocytobarines are specific for the bacteria. After impairment to the living functions the bacteria, like the spirochetes and trypanosomes, lose the capacity to adhere to the blood platelets in the presence of thrombocytobarine.

ROY C. AVERY.

ISOLATION OF SERUM PRECIPITINS. GONZO SUNOUCHI, Arb. a. d. med. Universität zu Okayama **1**:1, 1928.

Precipitin antiserum against beef serum is treated with some dried, powdered antigen. The precipitate formed is removed by centrifugalization, washed with physiologic solution of sodium chloride, suspended in distilled water or physiologic solution of sodium chloride and then tested with beef serum. The highest titer is obtained when the precipitate is shaken with water for from one-four to one-half hour at from 53 to 55 C., from which the author concludes that antigen-antibody precipitate is decomposed most completely under those conditions. The complement-fixation antibody is found with isolated precipitin, in equal amounts, from which the conclusion is drawn that precipitin and amboceptor are identical.

ARTHUR G. COLE.

Tumors

SUPERFICIAL EPITHELIOMATOSIS. HAMILTON MONTGOMERY, Arch. Dermat. & Syph. **20**:339, 1929.

According to Montgomery, superficial epitheliomatosis is a primary epithelioma of the skin clinically distinct. The characteristic lesion is single or multiple. It is a superficial, erythematous, scaling plaque with a rolled, threadlike, elevated border. Superficial epitheliomatosis is a frank basal cell, basal squamous cell or squamous cell epithelioma occurring usually on the trunk and as a rule having a benign course, but which may become malignant, metastasize and cause death. Data are given on a series of eighteen patients with this disease.

FRANK M. COCHEMS.

EXPERIMENTAL SARCOMA OF BONE. CHARLES L. CONNOR, Arch. Surg. **19**:794, 1929.

The author obtained a tumor of an endothelial structure which was found in a chicken by Dr. Murray, director of the Cancer Research Fund in London. The material was dried and suspensions of this were used in an attempt to reproduce the tumor in other chickens. It was found that this suspension gave rise to a rapidly growing tumor when it was injected into the thigh muscles in chickens. In this tumor it was not possible to determine its cellular origin, but it did, in general, have an endothelial structure and resembled in some instances the structure of Hodgkin's lymphosarcoma. When the suspension was injected into the bone-marrow, the tumor growth could be recognized on the fourth day by microscopic examination. Here, too, it was difficult to determine from which cells the

tumor was growing, although it did appear to rise from the undifferentiated cells of the bone-marrow. As the tumor progressed it grew up and down the medullary cavity, finally penetrating the bone to grow between it and the periosteum. The first gross evidence of tumor appeared on the fourteenth day. However, two of the chickens died before this happened, and they presented metastases in the liver and spleen. A parallel experiment was done using the Rous fibrosarcoma, and it was found that this tumor presented on the sixteenth day as a fusiform tumor similar to that formed by the endothelioma. The endothelial tumor was not an exact reproduction of that described by Ewing, since it possessed also some characteristics of an osteogenic sarcoma. It filled the bone-marrow cavity generally and seemed to possess an osteolytic power, but later, as it penetrated the bone and grew beneath the periosteum, it apparently stimulated bone formation. Bone formation occurred in the Rous tumor as well. In both instances, this happened when the cells were confined between the periosteum and the cortex of the bone. The author feels that the bone formation in this particular location indicates that endothelial cells and fibroblasts, when placed in a suitable physicochemical medium, may differentiate into osteoblasts.

N. Enzer.

Oligodendrogliomas of the Brain. Percival Bailey and Paul C. Bucy, J. Path. & Bact. **32**:735, 1929.

Proof has been obtained, by specific impregnations, of the existence of brain tumors composed essentially of oligodendroglia. These tumors are predominantly situated in the cerebral hemispheres of adults. They almost invariably contain sufficient deposits of the salts of calcium or iron to be visible in x-ray films. Oligodendrogliomas are slowly growing gliomas with a relatively good prognosis. The records of four such tumors are given in detail as well as data concerning nine other proved and four presumptive cases.

Authors' Summary.

Etiology and Pathology of Primary Lung Tumors. Norah H. Schuster, J. Path. & Bact. **32**:799, 1929.

Primary pulmonary tumors are becoming more frequent. There is no known etiologic cause. Sarcoma may occur. Primary thymic tumors may invade the lung by direct spread. The vast majority of tumors are epithelial in origin. The persistent presence of columnar cells and mucin in the various types suggest an origin from the bronchial lining or mucous glands. It is impossible to distinguish histologically between tumors that arise from bronchial epithelium and those from the glands and their ducts. The small cell tumors are epithelial in nature and probably arise from bronchial structures. The existence of alveolar carcinoma is not proved.

Authors' Summary.

Malignant Neuroblastoma of the Sympathetic. E. Bülbring, Virchows Arch. f. path. Anat. **268**:300, 1928.

A case of a tumor of the right suprarenal, the size of a child's head, with generalized metastases is described. It concerns a ganglioneuroblastoma connected with the medulla with remnants of the cortical tissue present. Numerous rosets were found.

K. Hosoi.

The Production of Tumor by Naphtha Products. G. Leitmann, Virchows Arch. f. path. Anat. **268**:566, 1928.

The author reports experiments on ten rabbits the ears of which were treated with a syrupy solution of toluene in petroleum. The changes were recorded six days, 20 days, two months, two and a half months, six months and eight months after the first application. The early changes were hyperemia, heat, swelling and loss of hair. In three animals, after six months papilloma and a cutaneous horn

developed. The stimulus or "x" substance in the toluene was regarded as having produced an epithelial proliferation of typical blastomatous nature. In all animals, cirrhosis of the liver was also found.

V. C. JACOBSEN.

HISTOLOGIC DIAGNOSIS OF CERVICAL CARCINOMA. W. SCHILLER, Zentralbl. f. Gynäk. **52**:1562, 1928.

Schiller described certain changes in the appearance and structure of the cervical squamous epithelium which he considers carcinomatous in nature and which he compares with Bowen's dermatosis. The transition between normal epithelium and pathologic epithelium is always sharp. As the diagnosis of carcinoma does not rest with definite morphologic changes nor on a histochemical test, he recommends staining the sections for glycogen with Best's carmine stain. The normal epithelium will show glycogen granules, while the pathologic epithelium will not contain any granules or only a few.

W. C. HUEPER.

TUMORS OF THE OMENTUM. E. M. FUSS, Zentralbl. f. Gynäk. **52**:1782, 1928.

Two cases of omental tumor are described. One was a primary sarcomatous endothelioma and the other an isolated metastasis of a papillary carcinoma of the ovary. Other metastasis was not present in either case.

W. C. HUEPER.

Medicolegal Pathology

INFLUENCE OF THE CADAVERIC MICROFAUNA ON DECOMPOSITION OF THE HUMAN BODY. C. F. PORTA, Arch. di antrop. crim. **49**:3, 1929.

This is a detailed study dealing with the classification and characteristics of various necrophagous species and their mode of destruction of dead human bodies, either exposed to air or interred, for the purpose of determining the time of death. The author discusses the practical value of the classification of the cadaveric fauna first attempted by Megnin in 1984; he reviews the additional entomologic investigations on this subject published since and classifies the known necrophagous parasites. Applying the postulates of Megnin, the study of the parasites present in the decaying body and the chronologic succession of the various insects allows one to determine the time of death, but only approximately. Of course, the meteorologic conditions also have an important influence on the disintegration of the cadaver. The necrophagous fauna varies according to the soil and climate. For instance, in stony soil, *Carabidae, Tenebriondae* and *Staphylinidae* prevail; in cultivated earth *Thanatophilus, Silpha* and *Acari* are frequently present; in forested or woodlands *Diptera* (of the genus *Lucilia, Muscina, Sarcophaga,* etc.) are common, while in swampy countries and along running waters *Necrodes* and *Cercyon* are often found. The investigations have disclosed that during the various stages of disintegration, certain characteristic insects are found: 1. During the chromatic stage *Diptera (Lucilia, Musca, Muscina)* and *Hymenoptera (Vespa)* appear. 2. In the second or emphysematous stage, *Coleoptera (Staphylinidae)* and *Diptera (Sarcophaga, Calliphora)* are observed. 3. During the first part of the colliquative stage (initial colliquation) *Coleoptera (Hister, Necrophorus Silpha, Saprinus, Thanatophilus)* are seen, while the second period of this same stage (terminal colliquation) is characterized by the appearance of *Coleoptera (Dermestes), Diptera (Phora, Cynomyia, Lonchoea, Anthomyia, Pyophila, Fannia, Scathopse, Ophyra)* and finally of *Lepidoptera (Aglossa).* 4. During the preskeletal stage *Acari* and several types of *Diptera (Thyreophora, Phora)* appear. 5. In the last period or stage of skeletization we meet *Coleoptera* of the genus *Anthrenus, Attagenus, Ptinus, Tenebrio, Tenebriodes, etc.,* a few *Lepidoptera (Tinea, Tineola),* and finally *Hymenoptera (Lasius, Solenopsis)* and *Thysanura.* Larvae may be encountered in any of the mentioned periods, which, of course, are not sharply defined; one may find, on one and the same cadaver, larvae and insects characteristic of various stages of decomposition. Only a predominance

of a certain species should be taken into consideration. The disintegrating action of the insects on a dead body exposed to open air, as to the sun in the summer months, in places in which the necrophagous fauna is richly represented, is about fourteen times as rapid as on cadavers found in cellars or rooms, or in enclosed or covered spaces, where the microfauna does not enjoy the optimal requirements for development.

E. L. MILOSLAVICH.

Technical

THE TECHNIQUE AND RESULTS OF A COMPLEMENT FIXATION TEST FOR THE DIAGNOSIS OF INFECTIONS WITH ENDAMEBA HISTOLYTICA. CHARLES F. CRAIG, Am. J. Trop. Med. **9**:277, 1929.

It is believed that the results of the complement fixation test for infection with *Endameba histolytica* demonstrate that substances occur in the blood serum of persons infected with this parasite, probably produced as a result of the reaction of the cells to the infection, which are specific in nature and which are capable of fixing complement when the serum is mixed with an alcoholic extract of cultures of this organism. It is not believed that this substance (or substances) is a true immune antibody. While the exact nature of the antibody is at present unknown, it certainly belongs to that group which is extracted with alcohol. Clinically, and as checked by the microscopic and cultural examination of the feces, the test would appear to possess a high degree of specificity, and its use in hospital practice has revealed amebic infections that would otherwise have remained unrecognized. The technic of this complement-fixation test is intricate and should be attempted only by a trained serologist.

JOHN PHAIR.

HYDROLYZED SERUM AGAR FOR THE ISOLATION OF CORYNEBACTERIUM DIPHTHERIAE. LUTHER THOMPSON, J. Infect. Dis. **45**:163, 1929.

A serum agar has been prepared by treating Loeffler's blood serum, dehydrated, with sodium hydroxide and incorporating the noncoagulable product in agar. The medium promotes the growth of diphtheria organisms, and inhibits certain other organisms found in the throat. It is easily available, easily prepared, can be safely sterilized in the autoclave and offers the convenience of an agar medium for the plating and isolation of pure cultures of *Corynebacterium diphtheriae*.

AUTHOR'S SUMMARY.

A SIMPLE METHOD OF MAKING CULTURES OF THE RECTUM. E. F. TRAUT and R. D. HERROLD, J. Infect. Dis. **45**:172, 1929.

Massage of the rectum with sterile petrolatum-coated finger cot or glove, followed by inoculation of its tip on to blood agar plates, gave cultures of streptococci and other bacteria in larger numbers as compared with the colon bacilli than could be secured by making cultures from the feces. The persistence of similar bacteria on repeated culture would indicate that the organisms found are not accidental saprophytes. A comparison of the fermentations by the streptococci isolated on successive culture is further evidence of the reliability of the results. The method offers an easy means of studying the relations of pathogenic bacteria of the rectum to disturbances of the intestinal tract itself, and also to arthritis and other systemic diseases.

AUTHORS' SUMMARY.

STUDIES ON ANTIGEN FOR THE KAHN TEST: PROPERTIES OF SENSITIZED ANTIGEN. G. LUBIN, R. L. KAHN and M. B. KURTZ, J. Infect. Dis. **45**: 196, 1929.

Sensitized antigens yield more sensitive reactions than standard antigen with syphilitic serum and cerebrospinal fluid. The sensitiveness is at a maximum

when the antigen contains an optimum proportion of sensitizing reagent and the total concentration of extractives is suitably adjusted. A further increase in the proportion of sensitizing reagent results in a decrease in sensitiveness. Sensitized antigens require a larger proportion of salt solution than standard antigen to give dispersable suspensions. Within certain limits the titer increases with the amount of sensitizing reagent present, reaching a maximum value which remains practically independent of further addition of reagent. Sensitized antigens are more susceptible than standard antigen to changes in the sodium chloride concentration and the p_H of the salt solution with which the alcoholic extract is mixed, and are more rapidly affected by exposure of the extract to sunlight.

AUTHORS' SUMMARY.

A PRECIPITATION TEST FOR SYPHILIS. EMIL WEISS, J. Infect. Dis. **45**:225, 1929.

The efficiency of the proposed test is based mainly on the concentration of reagents. The new method has the following advantages over the Kahn test: The dilution of the antigen is simpler and requires less time. The titration of the antigen is more accurate, being based on the reaction with serums or spinal fluids, respectively. The preparation of the antigen-serum mixture is simpler. The spinal fluid is added directly to the antigen dilution, thus avoiding the complicated procedure of concentrating globulins. The precipitation is produced by the combined effect of lipoids and sodium sulphate, and is very definite. The reading is facilitated by the higher dilution of the antigen-serum mixture at the conclusion of the test. The reading of results is more accurate and better defined. The entire routine test and the quantitative procedure are greatly simplified. The test gives a higher percentage of results agreeing with the results of the Wassermann test.

AUTHOR'S SUMMARY.

CATHETERIZATION OF THE RIGHT SIDE OF THE HEART. W. FORSSMANN, Klin. Wchnschr. **8**:2085, 1929.

The author describes the insertion into his own median basilic vein of 65 cm. of a ureteral catheter, and demonstrated by roentgen examination that the tip of the catheter extended into the right auricle of the heart. Dextrose solution containing other medicaments was given intracardially in this way to a moribund patient with apparently some beneficial effect. This method may be useful in studying metabolism and in investigating the functions of the heart.

EDWIN F. HIRSCH.

Society Transactions

NEW YORK PATHOLOGICAL SOCIETY

Anniversary Meeting, Jan. 23, 1930

HARRISON S. MARTLAND, *President, in the Chair*

BILE ACIDS, BILE PIGMENT AND CHOLESTEROL OF A, B AND C FRACTIONS OF BILE. J. CARLIND BROWNE, I. KATAYAMA and J. A. KILLIAN.

Bile was aspirated from the duodenum before and after the administration of magnesium sulphate. The siphonage of bile obtained after the administration of magnesium sulphate was divided into A, B and C fractions according to the technic of Lyons. Observations were made on thirty-five satisfactorily fractionated specimens from ten patients with chronic cholecystitis whose icterus indexes varied from 5.8 to 9.4.

Total solids, magnesium, bile pigment, bile acids and cholesterol were determined in the fractions of bile. The bile acid concentration of B bile was from two to ten times greater than that of A and C. Bile pigment in B bile was from four to twenty-five times greater than that of A and C bile. The cholesterol of A and C was uniformly less than that of B bile. A comparison of the magnesium of the A, B and C fractions of bile with the magnesium content of bile before the administration of magnesium sulphate has made it possible to compute the probable dilution of the bile with magnesium sulphate solution. These calculations indicated that the magnesium sulphate solution formed from 20 to 53 per cent of the A bile, from 16 to 23 per cent of the C bile and from 10 to 20 per cent of the B bile. It appeared significant that the total solids of B bile were definitely greater than the total solids of A or C bile, although the A and C fractions contained more magnesium sulphate. When the analyses of the bile fractions were corrected for dilution with magnesium sulphate solution, it was found that the bile pigment of B bile was twelve times greater than that of A, and three and four-tenths times greater than that of C; the bile acids of B bile were eight and one-half times their concentration in A, and three and seven-tenths times their concentration in C.

Comparisons have been made of the concentrations of bile pigment, bile acid and cholesterol of the bile fractions with their concentrations in the blood of the subjects drawn at the time of the biliary drainage. In these comparisons the concentrations of the three components of the bile have been corrected for dilution with magnesium sulphate. The cholesterol content of the A and C fractions was much less than that of blood, but the B fraction had a cholesterol content about twice that of blood. The bile acid and bile pigment content of the three fractions was definitely greater than that of the blood serum.

CHLORIDES OF GASTRIC CONTENTS IN HYPOCHLORHYDRIA AND HYPERCHLORHYDRIA. KATHERINE EYERLY and JOHN A. KILLIAN.

Free hydrochloric acid, total acidity, inorganic chlorides and total chlorides have been determined in specimens of gastric contents aspirated at intervals of fifteen minutes for two hours from patients who had received a salt-free meal, as described by Rehfuss. Results have been expressed in terms of milli-equivalents of chlorine per cubic centimeter. Graphs were presented to illustrate the principles of interpretation of the results.

Synchronous graphs for total chlorides and inorganic chlorides with curves for free hydrochloric and total acidity furnish data on the mechanisms producing achlorhydria or hypochlorhydria, i. e., the neutralization of gastric acidity by the ingestion of food, regurgitation of duodenal contents or a deficiency in secretion of hydrochloric acid. In the study of the production of hyperchlorhydria, these graphs may be utilized as indicators of hypersecretion, hyperperistalsis or a lack of neutralization.

A Case of Fulminant Infection with the Pfeiffer Bacillus. Adele E. Sheplar, Lawrence Sophian and Ward J. Mac Neal.

This case is presented in abstract because of several unusual features. In the first place, the clinical course was unusually rapid and led quickly to a fatal issue. In the second place, it was possible to demonstrate that the inciting organism was the Pfeiffer bacillus, and the organism was demonstrated in the respiratory tract, the lungs and the brain.

The patient was a boy, aged 3½ years, who had been apparently well up to about 2 p. m. on December 16. At that time he began to complain of feeling ill and was put in bed. The temperature reached 106.5 F. that evening. During the night, severe convulsions took place, and the patient died in a convulsion about 9 a. m. the following day. Autopsy was performed at 3 p. m. The positive observations were: a hemorrhagic injection of the mucosa of the trachea and bronchi, a moderate hemorrhagic injection in scattered foci in the lungs in all the lobes, and, in the meninges, a definite fibrinopurulent exudation over the cortex and base of the brain, extending down into the spinal meninges as well. The ears were normal. A smear of the meningeal exudate made at autopsy revealed a large number of polymorphonuclear leukocytes, and in and about these were seen abundant bacteria which ranged from small rod-shaped to fairly rounded coccoid forms. These were all gram-negative, staining very faintly with the counter stain. The organisms were mainly inside the leukocytes. A smear made from the injected foci in the lung showed a moderate number of pus cells, some of which were seen to contain gram-negative rods. There were also occasional groups of bacteria outside the cells. A culture of the spinal fluid was made on chocolate blood agar which within twenty-four hours showed very faint delicate colonies that did not grow on plain agar but that grew more luxuriantly in subculture on chocolate blood agar. These colonies consisted of pleomorphic gram-negative bacilli. Intraperitoneal injection of these cultures into mice did not kill the animals.

Microscopic examination of the meninges showed an extensive, purulent exudate filling the subarachnoid space, and some of the polymorphonuclear leukocytes invaded the superficial portion of the cortex of the brain, which was extremely edematous. Sections of the primary bronchi showed extreme engorgement of the capillaries of the mucosa and submucosa. Rather numerous mononuclear and polymorphonuclear leukocytes were noted between the epithelial cells and in some places on the surface of the mucosa in the lumen of the bronchioles. About the bronchioles the exudate was found to extend into the alveolar walls, separating the distended alveoli from one another by masses made up largely of red blood cells together with some mononuclear and polymorphonuclear leukocytes. The exudation was therefore within the alveolar walls rather than within the air spaces. The sections were stained with a modification of the eosin and methylene blue (methylthionine chloride, U. S. P.) stain in which, instead of methylene blue, azure B was used. This gave a powerful stain, and the bacteria could be seen stained dark blue or bluish purple in and about the leukocytes, in the bronchi, in the alveolar walls and in the meningeal exudate.

From this demonstration of the identity of this bacillus and its presence in the tissues in a case in which it appears to be the primary invader, we apparently have a new bit of evidence in regard to the potentialities of the Pfeiffer bacillus in producing disease under appropriate conditions.

DISCUSSION

WARD J. MAC·NEAL: I regard this case as of considerable importance because of the prolonged controversy that has existed since 1918 in regard to the etiology of epidemic influenza. We are not in a position to say that this is a case of epidemic influenza. We are in a position to say that it is an instance of a fulminating infection with the Pfeiffer bacillus. During the postmortem examination, before the cranium had been opened, the appearance of the bronchi and of the trachea was such as to cause the prosector to remark, "This is the sort of influenza we saw in France in 1918." The invasion of the meninges by the same organism, which has been demonstrated in the walls of the pulmonary alveoli, indicates that this organism was the important factor in the death of this child, who was, at 3 o'clock on the previous afternoon, actually playing outdoors. We can still afford to pay attention to some of the things we knew about influenza previous to 1918.

SOME OBSERVATIONS OF THE BACILLUS OF DUCREY. ADELE E. SHEPLAR and WARD J. MAC NEAL.

The discovery of Ducrey, who showed the presence of a small hemoglobinophilic, gram-negative, thread-forming, cylindric microbe in soft venereal sore, has been widely confirmed, and there is no longer any serious question that this organism causes a definite type of chancroid. However, the bacteriologic studies of soft chancre in various parts of the world have in some instances failed to disclose bacteria of this type, and on that account the specific relation of the bacillus of Ducrey to the common forms of chancroid and to chancroidal bubo have been questioned and in many clinics the Ducrey bacillus has been frankly disregarded.

The modern bacteriologic technic for chancroid has been developed by Teague and Deibert in America, Saelhof in England and especially by Paul Durand in Tunis.

For the last two years the presenters have employed a simple technic, essentially that of Durand, in making cultures from soft venereal ulcers. From 15 to 20 cc. of blood is taken from the elbow vein of the patient and most of this is distributed to miniature tubes in amounts of 1.5 cc. each, a portion being reserved for the usual serologic tests for syphilis. The tubes are slanted and left undisturbed until firm clot has formed. The sore is then carefully cleansed with cotton moistened with salt solution, a somewhat painful ordeal for the patient. Minute portions of exudate and of granulation tissue are removed from the base of the ulcer, preferably by means of sterile glass capillaries with cutting edges. These bits are seeded in the serum on the surface of the clotted blood. At least one such specimen of fresh material is smeared and stained for direct microscopic study. The culture tubes are placed at from 35 to 37 C. in the slanted position. After twenty-four hours, microscopic examination of the exuded serum on the clot usually reveals the bacteria, but the transfer of a large drop of this serum, by means of a sterile glass pipet, to the surface of a slant of mixed blood agar results almost invariably in the development of typical colonies of the Ducrey bacillus and usually without admixture of contaminating bacteria on the blood agar slant. After isolation the cultures remain viable in stoppered tubes of human blood agar at 35 C. and require transfer at intervals of one month.

Autogenous vaccines prepared from such cultures have given excellent clinical results in a small series of cases.

DISCUSSION

JAMES EWING: What is the histology of these lesions?

WARD J. MAC NEAL: We have not had enough material to make histologic examinations. The piece we obtain is only 0.5 mm. in diameter.

HARRISON S. MARTLAND: In your studies of the Ducrey bacillus in chancroids, can you identify the organisms in the dark field examination, or even suspect the infection?

WARD J. MAC NEAL: No, I do not think so; they are seen there as little dancing particles.

THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE BEHAVIOR OF ESCHERICHIA COLI AND ITS SPECIFIC BACTERIOPHAGE IN URINE. FRANCES C. FRISBEE and WARD J. MAC NEAL.

The colon bacillus grows well in human urine in a p_H range from 5.8 to 7.2 and most abundantly in the range from 6.5 to 6.8. The lytic action of bacteriophage added to such cultures in urine was most effective in the p_H range from 5.6 to 6.3, with a sharp inhibition of the lytic action at p_H 5.3 and a more gradual diminution of the lytic action toward the alkaline side beyond p_H 6.3. Exclusion of air appeared to be without significant influence.

Formaldehyde in a concentration of 1 per cent prevented visible growth of the colon bacillus in urine. In a concentration of 0.1 per cent, the bacillus grew and the lytic action of the phage was inhibited. Slight inhibition of the phage was evident in cultures containing one part formaldehyde per hundred thousand, but was not clearly discerned in a formaldehyde strength of 1 per million in urine.

Acriflavine, 1:1.000, inhibited the growth of the colon bacillus in urine. At 1:10,000 it allowed growth of the bacillus and inhibited the phage, especially in a p_H of 6.5 or less. Even at 1:1,000,000 a slight inhibition of the bacteriophage could still be recognized.

It would therefore appear that the treatment for infections of the urinary tract due to the colon bacillus by use of bacteriophage may be favored by maintaining a slight acidity of the urine. It is also evident that the formaldehyde and acriflavine are unfavorable to the action of the bacteriophage.

DISCUSSION

WARD J. MAC NEAL: This work makes no pretense of having any bearing on fundamental scientific questions. It is an attempt to study applied science, applied to a particular medium and in relationship to a particular type of human disease. However, when one starts out to investigate such a practical problem one sometimes finds evidence of general scientific value. It is evident that the two antiseptics tested exert a more powerful effect on the lytic agent than they do on the bacterial culture which is to be dissolved. Whether one can draw any definite conclusions as to the nature of the bacteriophage from such an observation I am unable to say, but it is true that we know of many enzymes which are capable of acting in the presence of an antiseptic, which is able to suppress entirely the cells producing such enzymes. Our observations would therefore tend to support the conception that the bacteriophage is a particulate living agent rather than an enzyme produced by the bacteria.

THE RELATION OF THE EPITHELIUM TO THE MUCOSA IN PACHYDERMIA LARYNGIS (WAX MODEL). LOUISE H. MEEKER.

The practical necessity of making a diagnosis from small bits of tissue rarely more than a few millimeters in diameter has stimulated this investigation. Sections from the larynx at various ages have been the basis of the study.

Vocal cords and their attachments have been dissected from persons aged 2 and 4 months and 2, 4, 11, 36 and 80 years, respectively, and coming to autopsy with no history of laryngitis. This material was cut in horizontal and vertical transverse and sagittal sections. The arrangement of the epithelium and mucous glands about the vocal process of the arytenoid and the elongation of the gland ducts with the ingrowing squamous epithelium seemed distinctive of this area, the area including the folding of mucosa adjacent to the vocal process. The notable points were the great activity of the squamous epithelium, with the rapid filling in of the wide ampulliform surface openings of the ducts with fusion into solid masses

at all ages. Within the deeper gland ducts, papillary thickenings of the epithelium, as well as a tendency to metaplasia, were very common.

The next step was the application of these pictures to frankly pathologic hyperplasia to ascertain when a diagnosis of a malignant condition was justified. The accredited signs of malignancy were checked off one by one in a selected example of pachydermia laryngis that we had considered on the borderline between a benign and a malignant process. The tissue was both nodular and diffuse and had been removed from a man, aged 39 years. In this case greater activity of the epithelium was found than was seen in the normal mucosa. We also found larger and more numerous papillae. Ingrowing of the squamous epithelium in the ampullae of the gland ducts and increase in the number of layers were noticeable, but atypical forms of cells were scarce. We found metaplasia and fusion in these areas with but little epithelial activity. Deeper in the glandular ducts we found changes all the way from papillary tufts to metaplasia of a few cells to metaplasia *en masse*, also increase in layers and fusion to form deeply placed islands. The proliferative activity was less here than near the surface, and the metaplasia followed the usual mode of transition seen normally on the surface. The activity of the surface epithelium together with the large and wide papillae indicated the third step in the study.

A nodule, showing many irregular papillae and numerous mitotic figures in microscopic sections, was chosen for a wax model. Fifty sections cut 10 microns thick in series were magnified 100 times by the Edinger apparatus. The numerous mitotic figures were marked wherever they occurred in a section by placing an opaque bead for each in the wax replica. The sections averaged 30 mitotic figures each, totaling 1,600 for a sector measuring 2.5 mm. in diameter and 0.5 mm. in thickness. The uppermost section showed 40 mitotic figures. The presenter depended on this method to discover whether there were any isolated foci of proliferative activity. The completed model was photographed, and x-ray pictures were made. The mitotic figures were seen evenly distributed at all points and in all layers.

The presenter compared the pathologic epithelial hyperplasia seen in a case of pachydermia laryngis, a "borderline case," with specimens of practically normal mucous membrane from the vocal process and posterior commissure.

The microscopic slides and the wax model studied somewhat lessened the seriousness of the first diagnosis.

DISCUSSION

JAMES EWING: Dr. Meeker's method is most ingenious and offers a means of study of many features of beginning carcinoma. The results obtained in pachydermia laryngis must make one extremely cautious in pronouncing an opinion concerning the clinical course of laryngeal lesions.

PAUL KLEMPERER: I think that we should be extremely grateful to Dr. Meeker for her investigations, because they emphasize the importance of great caution in diagnosing small fragments of laryngeal tissue and of making the diagnosis of carcinoma, only when we have all the criteria of carcinoma present. We should apparently not stress the possibility of carcinoma in a case in which we find downgrowths of epithelium alone, even with a considerable number of mitotic figures. Such a study, therefore, is extremely valuable because it confirms the more vague experience that all who do practical pathology encounter, and is helpful in our practical work.

ALFRED PLAUT: I think that the demonstration has practical and theoretical importance, not only for the larynx, but for other parts of the body where biopsies are obtained. These pictures remind one of the more or less atypical changes in the epithelium of the uterine cervix when the diagnosis on small particles of tissue has to be made. If such pictures are found frequently in autopsy material in which there is no suspicion of existing or developing carcinoma, then we have to be more and more careful concerning the term "precancerous." I never use

the term. Many people use it, and the people who do so certainly would use it for the pictures which Dr. Meeker has shown. In the uterine cervix we know that such pictures must not have anything to do with carcinoma. The larynx and the uterine cervix have one thing in common, i. e., that different kinds of epithelium are together in very small spaces, and at the same time are subjected to much mechanical irritation. There may be some other parts of the body where these conditions are true, so aside from the practical importance, which was stressed by Dr. Klemperer, I think that it gives us an idea of the usefulness, or nonusefulness, of the theoretical term precancerous lesions.

HARRISON S. MARTLAND: For some time I have been of the opinion that the occurrence of a few mitotic figures should not be considered absolute proof of malignancy. These figures often occur in tissues like the endometrium, which is constantly undergoing profound changes in structure. The popular tendency to grade tumors on the appearance of one or a few isolated cells is risky indeed and often misleading. This accounts for the great increase in the so-called precancerous lesions and the so-called malignant changes in simple, benign, gastric ulcers. For these reasons, I believe that Dr. Meeker's painstaking studies are of considerable importance.

PAUL KLEMPERER: Dr. Plaut's remark recalls to my mind a case of tuberculosis of the lungs in which I made routine sections of the larynx looking for tuberculosis. To my surprise I found the most remarkable proliferation of the epithelium, even with irregularity of the cells, but on macroscopic reexamination of the larynx I could not find any evidence of tumor. In cases of tuberculosis, one has to be particularly conservative.

A RECONSTRUCTION STUDY OF THE FOLLICLES AND FOLLICULAR ARTERIES OF THE HUMAN SPLEEN. WARD J. MAC NEAL and J. M. RAVID.

From earlier studies it has been concluded that the splenic circulation is essentially open, in the sense that the elements of the blood pass from the arterial terminations into the intercellular spaces of the pulp and thence through the stomas of Mollier into the venous sinuses. It was further concluded that the functional unit of splenic structure, or the splenic lobule, may be recognized as consisting of a central follicle, a bordering marginal zone and the radiating pulp cords. In a perfused specimen of spleen, the lobule is outlined by the most widely distended venous sinuses at its periphery.

The present study is concerned with a small terminal branch of the splenic artery and the splenic follicles supplied by it. The spleen of a child perfused and fixed in the distended state was used for a series of stained sections cut at a thickness of 5 microns. These sections were then projected and drawn at a magnification of 20 diameters. The outline drawing of every tenth serial section was transferred to a wax plate 1 mm. thick, and the cut wax plates were fitted together with suitable wire support to form a wax model.

The model shows the follicles in relation to their special arterial vessels in a region including the external capsule of the spleen and extending about 10 mm. beneath it into the splenic substance. The follicles more deeply situated are irregularly spherical and possess a single terminal artery of considerable length which enters the follicle and divides to supply only the lobule pertaining to that follicle. The coarser arterial twigs running out into the pulp carry sheaths which appear to be prolongations of the follicular substance, indicating that the ellipsoid sheaths are possibly of the same nature as the lymphatic tissue of the follicle.

Near the surface of the spleen, however, the follicles tend to be more elongated and narrow and tend to stretch along one side of an arterial branch which may continue on beyond to supply a follicle situated nearer to the external capsule. The appearance suggests that these lobules near the capsule may be less mature than those deeply situated. They still present the appearance of compound lobules of irregular shape, the individual follicles being not yet completely surrounded by large venous sinuses so as to define the lobule completely.

DISCUSSION

PAUL KLEMPERER: I would like to ask one question. It seems from the model, particularly of the areas distant from the capsule, that the lymphatic tissue around the arteries is accumulated in the form of follicles only. In the portion nearer the capsule the lymphatic follicles seem to be elongated, more cylindric. I wonder if this elongation does not indicate that there is also, between the follicles, a lymphatic sheath around the artery. I remember the article and pictures of Hellsten (*Ztschr. f. mikr.-anat. Forsch.* **13**:43, 1928). He states that in human spleens there is always a lymphatic sheath around the artery between those portions of the vessel which are surrounded by a distinct follicle. I think that it is different in rabbits. The lymphatic tissue is arranged in continuous cylindric sheaths with only insignificant follicles. I remember that the cat was the third mammalian examined and there, if I am correct, conditions are similar or identical with those in human spleens. I should like to ask whether Dr. Mac Neal did not find any lymphatic tissue arranged in cylindric sheaths between the follicles, though in moderate amounts.

WARD J. MAC NEAL: I am not sure that I appreciate Dr. Klemperer's question. It is, of course, true that lymphatic sheaths occur about the vessels between the follicles, but it is not quite clear to me what type of vessel is referred to in the question. The arterioles of the pulp cords are surrounded in part by lymphatic sheaths, and the ellipsoids of Schweigger-Seidel are possibly of a similar nature. Without reconstruction studies such vessels may possibly be mistaken for larger trunks. As far as lymphatic sheaths for the larger arteries, which I would designate as intralobular arteries, those that I have spoken of as stems of the cherry, they are surrounded by venous pulp and do not possess a sheath which contains lymphoid cells. They have an ordinary arterial wall with a little adventitial tissue. They enter into the follicle in an abrupt fashion.

The follicles that are distributed along the arterioles are not found near the external capsule of the spleen. The significance of this arrangement is not clear to me. It is a question whether these represent developing portions of the spleen, analogous to the developing cortex in the kidney of a young child, or whether they represent fully developed lobules of a peculiar form. There is also the possibility of other explanations. It will be necessary to study other spleens before we decide.

PAUL KLEMPERER: Of course, I meant the arteries of first magnitude, and I wonder if the model which was shown is not the exception to the rule. I think that the paper by Hellsten would indicate it.

WARD J. MAC NEAL: Did he use perfused spleens? When you perfuse a spleen you need examine only a single section to find intralobular arteries without lymphatic sheaths.

HISTOLOGIC STUDY OF SACROCOCCYGEAL CHORDOMA. LAWRENCE SOPHIAN and NICHOLAS M. ALTER.

This report is added to the approximately seventy cases already in the literature because of the relative rarity of this type of tumor. There is, however, still considerable confusion in regard to the pathogenesis and histologic nature of this lesion (other cases with similar histologic pictures have been described as myxochondroma, spindle cell sarcoma, colloid carcinoma, etc.). Therefore, an attempt has been made to apply a variety of stains with the object of making as complete a histologic analysis of the tissue as possible.

A man, aged 44, entered the New York Post-Graduate Hospital complaining of pain in the back of two years' duration. Shortly after the onset he noted a lump in the sacral region. At the time of admission this lump had reached the size of a football, and there were also difficulty in defecation, frequency of urination and some incontinence. There was no bleeding into the rectum. Destruction of the lower portion of the sacrum and coccyx was seen by roentgen examination. An attempt was made to remove the growth, but two hours later the patient

died suddenly with severe pain in the precordium. This change was considered clinically as pulmonary embolism.

The pathologic specimen consisted of a growth weighing 7 pounds (3.2 Kg.), with an outside surface partly covered by skin, having an area 21 by 7 cm. The mass showed partial encapsulation with numerous lobular subdivisions, some of which were transparent and jelly-like; others were fibrous, and still others, cystic. There were also numerous hemorrhagic foci. There was a considerable amount of fibrous tissue, some of which was loose; other portions were extremely tough and tendinous. Toward one surface numerous fragments of bone, apparently portions of the sacrum, some of considerable size, were noticed. Numerous sections from various parts of the tumor were studied and stained for evidence of metabolic and degenerative products. A great variation of cell type in different parts of of the tumor was noted. In some foci there were many vacuolated physaliphorous cells in a loose syncytial arrangement. Elsewhere the tissue was dense with bundles of collagen about rather small compressed cells. Here the resemblance to scirrhous carcinoma was marked. The presence of glycogen within the large vacuolated cells has been noted by other authors. In the case presented the material was not available for this examination. Within and between the cells could be found a material staining with mucicarmine and also with the toluidine blue method for mucin recently described by R. J. Lillie. No material staining with sudan III or scarlet red could be demonstrated. By picrofuchsin, phosphotungstic acid-hematoxylin and Mallory's aniline blue stain, the presence of fibroglia and collagen fibrils could be determined between the cells of the tumor. These fibrils did not appear in relation to supporting stroma connective tissue but seemed rather to arise from the tumor cells themselves. The appearance suggests that these cells are an embryonal variety of fibroblasts secreting a fluid substance which expands the cells and then breaks out between them. When in the intercellular spaces this fluid apparently jells and becomes a fibrillar material which appears to be identical with collagen in staining characteristics. While the identification of the substance is not complete, it appears from the present evidence that the cells called chordoma cells are probably really embryonal cells capable of manifold differentiation, which secrete a substance that may become converted into collagen.

DISCUSSION

BORIS KWARTIN: I should consider the presence of mucin a sign of degenerative changes in the neoplasm rather than a primary property of the cells, because the chorda cells proper in the nucleus pulposus, as well as the physaliphorous cells in the chorda dorsalis of the embryo, are free from mucin.

LAWRENCE SOPHIAN: I cannot answer Dr. Kwartin's question exactly, but at least it is suggestive that mucous connective tissue is embryonal; the connective tissue that is seen in embryos and in the umbilical cord normally, when a question of degeneration does not come into play, is of this type. The occurrence of mucoid degeneration in connective tissue is seen in a great many other regions, and I have found areas of degeneration in which the presence of mucinous material cannot be questioned; however, when we are dealing with a tumor of embryonal origin, we should not be surprised to see that an embryonal variety of intercellular substance is also present.

NICHOLAS M. ALTER: I have been interested in embryonal tumors for a long time, particularly from the standpoint of the intercellular substance as well as the intracellular secretion, both of which are often identical with substances found during degenerative processes in pathologic conditions. They are evidently due to changed cell metabolism, for example, the amyloids. Chemical analysis and study by special stains will also reveal a great variety of this substance, although all these varieties are probably related to chondroitin sulphuric acid. About two years ago I had the opportunity to talk before the Society on lipoblastoma. In these embryonal fatty tumors I also found a variety of intercellular substance that is also present in many other tumors, which are called simply

myxoma. In some types of malignant chordoma, atypical amyloid, mucoid, collagenous and other interstitial substances are present.

I also had the opportunity to observe some cases of chordoma clinically for a considerable time. They have a long-continued benign period, after which they take a rapidly growing malignant course. These tumors probably occur more frequently, but they are often confused with other tumors; they are often called benign chondroma or chondrosarcoma, or sarcoma of the pelvis.

HARRISON S. MARTLAND: The tumor in question looks more like a chordoma than a great many cases of that disease reported in the literature. Consider the myxomas of the bladder; it is a question whether they are not just ordinary edematous fibroblastomas and have nothing to do with mucin—Wharton's jelly.

NEOPLASM DERIVED FROM MUCUS-SECRETING CYLINDRIC CELLS OF THE LINING OF THE URINARY TRACT. NICHOLAS M. ALTER and JOSEPH F. MCCARTHY.

Mucus-secreting cylindric cells can give rise to unusual neoplasms along the urinary tract. Two such cases furnish the material for the present study. One was malignant, a so-called mucoid carcinoma of an exstrophic urinary bladder; the other was benign, a mucinous adenocystoma of the pelvis of a horseshoe kidney. Both of these cases were from the service of Dr. Joseph F. McCarthy at the New York Post-Graduate Medical School and Hospital.

CASE 1.—I. S., a white man, aged 51, had been under observation for eighteen years. He was admitted to the Post-Graduate Hospital sixteen times in the interval from October, 1919, to Oct. 3, 1928. Previous to admission he had had nine operations for attempted closure of the bladder. These closures, however, reopened from time to time. There were symptoms also of mild infection of the urinary tract. In 1922, the patient began to have hematuria; the fistula of the bladder became everted and the edges were covered with fungoid growth. The growth was partly excised, and the patient was treated with diathermy. Pathologic examination of this tissue revealed a papillary adenocarcinoma. In November, 1923, Dr. McCarthy performed an operation for total cystectomy and left ureterostomy to the anterior abdominal wall. On Dec. 20, 1924, as no renal function but purulent discharge was noted from the right ureter, Dr. McCarthy performed a left nephrectomy, without the removal of the corresponding ureter. The patient's last admission was on Sept. 18, 1928, at which time he complained of headache, general weakness and drowsiness. At that time the chemical analysis of the blood showed: urea nitrogen, 41.6; creatinine, 5.7. The patient died on October 3, with symptoms of uremic acidosis. Autopsy was performed twelve hours later. The anatomic observations were confined essentially to the urinary tract. There was a crater-like ulceration of the anterior abdominal wall corresponding to the urinary bladder. The edges of this crater were everted. The lining of the crater consisted partly of mucoid, jelly-like material and partly of yellowish-green, necrotic material. The interval between the pubic bones was 13 cm. The penis measured 37 mm. in length. The dorsal surface was partly invaded by the crater-like ulcer. About 9 cm. above the growth, somewhat to the left, an opening of the left ureter was seen. The left ureter measured 12 mm. in diameter. The right ureter was much distended, measuring 3 cm. in diameter. The lower end of the right ureter was invaded by the jelly-like growth. On section of the growth, a honeycomb-like structure was seen. There were also some cystic cavities which seemed to be filled with mucoid material. No normal mucosa was demonstrated. The edges of the growth were surrounded by skin. The left kidney was considerably and uniformly enlarged and weighed about 250 Gm. It was very firm in consistency. The capsule stripped off easily. On section a whitish, pale, rubbery parenchyma was seen. The iodine reaction showed brown discoloration with specks and streaks. The renal pelvis was slightly distended. The mucosa was hemorrhagic and covered with turbid, greenish, purulent exudate. An encapsulated mass, not unlike a kidney in shape, was seen at the upper end of the right ureter. On cross-section, only a metastatic growth, 9 by 6 by 5 cm.,

was seen. On section, it showed irregular cystic cavities filled with grayish-white jelly-like material. Both lower lobes of the lungs contained irregular larger areas of consolidation. On section of these areas, grayish granular tissue was seen. The areas were not air-containing. The spleen weighed 180 Gm.; it was firm in consistency. On section, the typical picture of "sago" spleen was seen, with large mahogany-colored globules after the iodine reaction. Section of the edge of the crater showed the abrupt edge of a growth which consisted of smaller and larger cavities filled with amorphous secretion containing small round signet ring-shaped cells with peripheral nuclei. The secretion gave the microreaction of mucin with the special stain (thionine, polychrome methylene blue). Most of the cavities had no definite lining. The microscopic section of the lower end of the right ureter and of the mass at the place of the right kidney showed a typical picture. Sections of the left kidney showed advanced amyloid degeneration of the tubuli as well as of the glomeruli, which were filled with homogeneous masses of amyloid. In the spleen the amyloidosis was mostly restricted to the malpighian corpuscles. Sections of the lung showed bronchi and adjacent air spaces filled with masses of polymorphonuclear leukocytes and red blood cells.

CASE 2.—Benign tumor of the renal pelvis of a "horseshoe kidney." V. C., a white man, aged 25, was admitted to the urological service of the New York Post-Graduate Hospital on Feb. 17, 1928, with a chief complaint of pain in the right groin and pyorrhea. Dissection of the pyonephrotic portion of the horseshoe kidney was performed by Dr. McCarthy. Grossly, this specimen had a bosselated appearance, covered with dense fibrous adhesions. It weighed 150 Gm. On section, the calices were cystically distended and filled with green purulent exudate. The upper calix contained a large impacted stone, forming a cast of the distended calix. A circumscribed growth, 35 by 25 by 13 mm., was situated at the upper orifice of the ureter. It had a honeycomb-like cystic structure and contained mucoid material. Section of the growth of the renal pelvis showed an abrupt change from the transitional epithelium to cylindric cells. The cylindric cells were mostly arranged in one layer. The cystic cavities were partly lined with these cells. In great part, however, no lining was seen. The cylindric cells were of the goblet cell variety, with marked mucoid secretion. The content of the cystic cavity, as well as the secretion of the cell, gave a typical mucin reaction (mucicarmine, thionine, polychrome methylene blue). All of the cells were of the typical differentiated mature type. No mitotic figures were found. The growth was well limited. There was a stroma which had irregular bundles of spindle-shaped smooth muscle cells, and there were larger blood vessels with thickened walls.

Tumors with mucus-secreting cylindric cells are rarely derived from the lining of the urinary tract. There are fifteen cases of mucoid carcinoma of the urinary bladder associated with exstrophy reported in the literature. Not a single case of circumscribed mucinous adenocystoma of the renal pelvis has yet been reported. Two explanations have been offered for the histogenesis of this type of neoplasm: (1) embryonal misplacement (Ehrich, von Gelder, Keith, Hache, Judd, Plaut, etc.); (2) metaplasia following inflammatory changes (pyelitis cystica) (Stoerck, Enderlen, Klemperer, etc.). In most of the cases reported embryologic malformations were present. In the first case presented, the mucoid carcinoma of the bladder was associated with exstrophy of the bladder, hypoplasia of the penis, undescended testis (left) and cleft pelvis. The second case presented was surgical and only the horseshoe kidney is recorded. Adenocarcinoma of the bladder is rare and is commonly associated with exstrophy of the bladder. Embryonal misplacement of the intestinal mucosa not only is possible in the urinary bladder, which is a part of the cloaca, but also is possible along the ureter, which buds out from the cloaca. In the second case, no pyelitis cystica was present. In the adenocystoma of the renal pelvis only high cylindric cells with mucous secretion were demonstrated. They were arranged in one layer. No cells of the transitional epithelium type were present. Inflammatory reaction may be responsible for the differentiation and hyperplasia of the dormant embryonal cells. If the inflammatory reaction and stone formation were entirely responsible for this tumor, it ought to be found frequently.

Regular Meeting, Feb. 27, 1930

LEILA CHARLTON KNOX, M.D., *President, in the Chair*

STUDIES IN ISOHEMAGGLUTINATION. ALEXANDER S. WIENER, MAX LEDERER and S. H. POLAYES.

In table 1 is presented the results of an analysis of the maternal blood and of the blood from the umbilical cord in a series of 500 cases. It will be seen that the blood from the cord has been classified under the four classic, fully established groups: O, α β; A, β; B, α; and AB, o; and five subdivisions, O, o O, α; O, β; A, o; and B, o. These five subdivisions do not represent any new

TABLE 1.—*Results of Analysis of Maternal Blood and Blood from the Umbilical Cord*

Group of Mother	Number of Cases Examined	Number of Children								Incompatibility	
		O, o	O, α	O, β	O, $\alpha\beta$	A, o	A, β	B, o	B, α	AB, o	Number of Cases of Cord Serum Agglutinating Cells of Mother	Number of Cases of Mother's Serum Agglutinating Cells of Child
A, β	200	26	0	25	0	70	50	19	0	10	0	29
O, $\alpha\beta$	187	25	11	5	72	22	25	11	16	0	0	74
B, α	84	11	7	0	0	16	0	12	31	7	0	23
AB, o	29	0	0	0	0	15	0	8	0	6	0	0
Totals	500	62	18	30	72	123	75	50	47	23	0	126

TABLE 2.—*Results of Examination of Ninety-Four Families in Which One of Parents Belonged to Group AB*

Group of Father	Group of Mother	Number of Families	Group of Children				Totals
			O	A	B	AB	
?	AB	69	0	43	27	19	89
0	AB	10	0	12	14	0	26
A	AB	5	0	0	1	4	5
AB	A	1	0	3	0	0	3
B	AB	7	0	3	10	4	17
AB	AB	2	0	1	0	1	2
Totals		94	0	62	52	28	142
Per cent			0.0	43.66	36.62	19.72	100.00

groups peculiar to the blood from the umbilical cord but incompletely developed blood groups. From table 1 it is seen that 217 of the 500 specimens of blood from the cord, or 43.4 per cent, presented fully developed groups. Of particular interest, however, is the fact that not a single case was found in which the mother gave birth to a child whose serum contained an agglutinin which could agglutinate the mother's cells. This is all the more striking since there were 126 cases in which the mother's serum could agglutinate the cells of the child.

A practical application of this observation is that in case of emergency a newborn child can be given a transfusion with the blood of its mother without previous grouping or cross-matching, as that would be analogous to the use of a universal donor. However, we would recommend always choosing a donor of the same group, particularly in repeated transfusions.

The Bernstein theory and the von Dungern and Hirszfeld theory of heredity of blood groups were discussed. One thousand three hundred and thirty-four mothers with 1,462 children were grouped in order to determine which theory was correct. Our statistical data prove the inaccuracy of the theory of von Dungern and Hirszfeld and fully support the Bernstein theory. In table 2 are

listed the results of the examination of 94 families in which one of the parents belonged to group AB. Not one of the 142 children in these families belonged to group O. Moreover, among 516 children of 485 mothers belonging to group O, not one child belonged to group AB. It is of interest to note that according to the theory of von Dungern and Hirszfeld, these combinations should have appeared in our series of cases more than thirty-four times. Our data therefore completely support the Bernstein theory and prove the inaccuracy of the theory of von Dungern and Hirszfeld. In view of the fact that Haselhorst and Kossovitch have found two cases that seem to constitute actual exceptions to Bernstein's theory, however, some slight modification of that theory may be necessary, such as postulating the occurrence of mutations or nondisjunction, or the existence of another factor.

The application of blood groups to medicolegal cases in which the identification of blood stains or the determination of paternity is necessary was discussed. The work of Landsteiner and Levine on the new agglutinogens, M, N and P was also discussed.

DISCUSSION

MAX LEDERER: It is indeed surprising that there has not been a greater application of this knowledge in medicolegal work. In talking with Dr. Bernstein two years ago, he told me that it was being employed extensively in Germany, especially in determining paternity. The use of the test has discouraged a great many accusations by women who have falsely accused men of being the father of their children, thus exposing them to perjury. Schiff, in an excellent review, emphasizes the value of the test in a case in which a person had been accused of murder, and in which the main evidence of the prosecution rested on some blood stains which were found on the clothes of the defendant. The latter claimed he had cut himself. The prosecution insisted that the blood was from the murdered man. Examination of the blood stains showed the blood to be in the same group as that of the defendant, and in a different group from that of the deceased. In speaking with several jurists on this point, they have all expressed a profound degree of ignorance on this subject, and, in an effort to popularize its value, we thought it would be of interest and value to present the work at this society.

ALEXANDER WIENER: When M and N, which have been recently described by Landsteiner and Levine, have been worked up sufficiently so that they can be used medicolegally with A and B, the chances of proving nonpaternity of an innocent man will be almost one of three. With A and B alone the chances are one of seven. Landsteiner and Levine are working on additional agglutinogens now, and in the future we may be able to recognize the correct parents in practically every case.

PHILIP LEVINE: With regard to Dr. Wiener's statement concerning the possibility of obtaining further instances of excluding paternity, there is new evidence as to the existence of many differences in human blood. This is derived mostly from serologic experiments with immune antihuman agglutinins and with atypical agglutinins found in a small number of human serums. More indirect evidence as to the individuality of human cells in general has been obtained from the results of transplantation, since it is known that only autotransplants are successful. Similar results for animal blood were already obtained by Ehrlich and Morgenroth, and more recently by Todd. The practical application of these results with human blood must await perfection of the serologic method.

CHRONIC ULCERATIVE COLITIS. REPORT OF VACCINE THERAPY. WILLIAM Z. FRADKIN and IRVING GRAY.

In a series of patients suffering from chronic ulcerative colitis the Bargen diplococcus was isolated from the colonic ulcerations in twelve of fifteen patients. Three failed to show the Bargen diplococcus after repeated bacteriologic study. Material for culture was obtained by inserting a long, sterile swab through the

sigmoidoscope and scraping the depths of an ulcer. This material was immediately transferred to warm dextrose broth and incubated for about six hours. This was then subcultured in other tubes containing dextrose broth, and finally litmus lactose and blood agar plates were poured with material from the last culture tubes. The typical colonies were fished out and transferred to warm dextrose broth. The diplococcus fermented dextrose, lactose, saccharose, maltose and occasionally mannite, but never inulin. It was not bile-soluble. Vaccine was made containing approximately two billion organisms per cubic centimeter. The organisms were agglutinated by the patient's serum in each case. Intravenous injections of a pure culture of the diplococcus into rabbits produced lesions in the large intestine similar to those found in the human being. The submucosa of the gut showed myriads of the diplococci.

After eight injections of the vaccine, ten patients showed immediate improvement. The number of stools decreased rapidly and a gain in weight was noted in each case. Vaccine therapy was continued in certain cases for more than four months. One patient revealed a pure culture of the Bargen diplococcus in nasal and tonsillar smears. Two patients did not respond to vaccine therapy. Improvement was noted more often and sooner and was more lasting when specific treatment was supported with rest, fresh air, small transfusions of blood, high caloric and high vitamin diets, and the irradication of all possible foci of infection.

DISCUSSION

M. A. KUGEL: Does this organism ferment esculin, and have you differentiated it from the ordinary enterococcus?

WILLIAM Z. FRADKIN: It has not been tried.

PERRY MANHEIMS: Were these organisms submitted to the heat-resistance test? In practically every case in which we have made examinations of the stools and proctoscopic examinations, we have found an enterococcus which corresponds to the Bargen bacillus in every way, except in regard to the heat-resistance test; the Bargen bacillus is not heat-resistant, while the enterococcus is heat-resistant. We have also found the Bargen bacillus in one instance in which there was no evidence of ulcerative colitis in practically pure culture.

WILLIAM Z. FRADKIN: We have not tried the heat-resistance test. The Bargen diplococcus differs from the enterococcus in that it is bile-insoluble and does not ferment inulin. We were not fortunate enough to find the diplococcus in practically pure culture in patients not manifesting any signs or symptoms of chronic ulcerative colitis. The object in presenting this work tonight was to report the marked improvement obtained in patients with chronic ulcerative colitis by vaccines prepared with the Bargen diplostreptococcus.

BILIARY AND HEPATIC FACTORS IN PEPTIC ULCER. BENJAMIN N. BERG.

Peptic ulcers were produced experimentally in dogs by excluding the flow of bile from the intestine. Three series of animals were studied. The first group consisted of seven animals with uncomplicated biliary fistulas. Acute duodenal ulcers were found in four of them. The second group included eleven animals in which biliary fistulas were complicated by biliary obstruction. Lesions were found in six animals. In three of these dogs, chronic duodenal ulcers were found; in one, a subacute perforated duodenal ulcer developed; in another, subacute ulcers of the duodenum and stomach were encountered; and in the sixth animal there were multiple erosions of the stomach and duodenum. The third group included five animals with complete biliary obstruction. Ulcers developed in three of the dogs in this series. In two animals, chronic duodenal ulcers were found, and in one there were multiple gastric erosions.

The relationship of environment and diet to the incidence of ulcers was studied. It was found that lesions developed more readily under unhygienic conditions and with poorly prepared diets.

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The relationship of environment and diet to the incidence of ulcers was studied. It was found that lesions developed more readily under unhygienic conditions and with poorly prepared diets.

The mechanism by which the exclusion of bile predisposes the mucosa to ulceration depends probably on the loss of some constituent, such as mucus, which is essential for the preservation of an intact surface epithelium.

The results of the investigation suggest the possibility that alterations in the function of the liver and in the secretion of bile may be important factors in the etiology of peptic ulcers. Although gross or microscopic changes in the biliary tract are found in only a small percentage of cases of peptic ulcer in man, nevertheless, functional disturbances which are not recognized by present inadequate methods may exist. The periods of remission and exacerbation that characterize so-called chronic ulcers may coincide with intermittent functional alterations in the stomach and duodenum in response to changes in the liver and in the biliary system.

DISCUSSION

DAVID PERLA: This is an extremely interesting and suggestive piece of work. It has been the observation of various investigators working with suprarenal glands in animals that gastric ulcers have commonly developed following bilateral suprarenalectomy. We have had occasion to observe 500 rats with bilateral suprarenalectomy, and gastric ulcers developed in a high percentage of these animals. These observations were made incidental to other work. It would seem to me from what Dr. Berg brought out that another explanation is possible to account for the appearance of gastric ulcers following surgical intervention in the biliary system. It is barely possible that in doing the operations they interfered with the nervous supply to the mucosa in those parts of the duodenum and stomach in which they found the ulcerations, and I would like to ask if that was carefully controlled. The same mechanism might hold true with the removal of the suprarenal gland, that is, an interference with the tonicity in regions in which these ulcers subsequently develop might effect the mucosa, with subsequent formation of erosions and ulcers.

PAUL W. ASCHNER: Ulceration of the stomach and duodenum has, of course, been produced by a great many procedures, but we do not know the cause of gastric and duodenal ulcer. Dr. Berg's observation that the condition of the animal, the hygienic conditions and the feeding made great differences in the incidence of ulcers is important, because it adds weight to the belief that general factors come into play in the production of ulcers. It is well known that prolonged exclusion of bile from the intestinal tract has an injurious effect on the general body economy. For example, grave anemias and also osteoporosis have been noted after prolonged biliary drainage. I wonder whether any observations were made in these animals of the effect on the blood and bones as a result of prolonged exclusion of bile from the intestinal tract.

If disease of the gallbladder, or of the biliary tract, has a frank influence in the etiology of ulcer, we would expect to find ulcer of the stomach and duodenum much more common in women than in men, but the reverse is true. We find disease of the gallbladder and biliary tract common in women, and gastric and duodenal ulcer common in men. During the last year we have had three women on the wards of Mt. Sinai Hospital who had coincident disease of the gallbladder and duodenal ulceration, so that it is possible in these particular patients that there may have been some relationship between the two conditions. Some experiments suggest themselves to my mind for definitely determining the rôle of the mucus from the gallbladder as a protective factor. Any information we may get concerning the etiology of ulcer is always welcome and interesting.

PAUL KLEMPERER: It seems to me that Dr. Perla's suggestion is a good one. I feel too that some controls should have been mentioned of operative procedures in the same region, but without the production of biliary drainage. It occurs to me that we see frequently on the autopsy table postoperative biliary fistulas and complete obstruction of the common duct, even with occlusion of the pancreatic duct of long duration, and we fail to see ulcers in these cases. I saw

only recently one case in which there was a biliary fistula and obstruction in the ampulla of Vater with obstruction of the pancreatic duct. In this case there were multiple acute ulcers of the stomach. I have reviewed, in connection with this case, our entire material, and I found that this was the only case of its kind. Unfortunately, in this instance there was a severe infection around the pancreas with thrombosis of the artery and splenic vein, and it is most probable that the ulcers of the stomach were circulatory in origin. It is surprising that we do not encounter such instances of gastric and duodenal ulcers in man in whom obstructions of the common duct are so frequent.

MENDEL JACOBI: I should like to ask Dr. Berg if he noticed any difference in the degree of occurrence of pericholangitis and abscesses of the liver in the group of animals kept under the poor conditions downtown and in those kept under the better hygienic conditions prevailing uptown in the new medical buildings?

BENJAMIN N. BERG: Although numerous experimental methods have been employed to produce peptic ulcers, few investigators have been successful in producing chronic lesions. The ulcers which develop after bilateral adrenalectomy are usually acute lesions. Operative trauma played little rôle in the experimental procedure because the common duct was isolated without disturbing any of the adjacent structures. When the common duct is divided, the nerves accompanying it are severed. However, this had no etiologic significance with respect to the ulcers which were produced, because a similar procedure was followed in transplantation experiments without the formation of ulcers. Ulcers were never observed after bilateral division of the splanchnic nerves or after extirpation of the celiac ganglions. Although mild anemias were noted after the establishment of biliary fistulas, the changes in the blood were not marked and were associated probably with alterations in nutrition. Osteoporosis was not observed. Peptic ulcers are found associated with lesions of the biliary tract in man in a small percentage of cases. However, this does not preclude the possibility of the interrelationship between disordered function of the liver and peptic ulcer. The function of the liver may be disturbed markedly without gross changes in the biliary tract. In a series of cases of biliary fistulas in man, which were collected at the Presbyterian Hospital, peptic ulcers were encountered occasionally. Infection of the liver was found frequently whenever biliary obstruction existed for long periods. In animals with uncomplicated biliary fistulas, infection was usually absent. There was no relationship between the occurrence of infection of the liver and the environmental conditions.

SUBACUTE BACTERIAL ENDARTERITIS OF THE PULMONARY ARTERY ASSOCIATED WITH PATENT DUCTUS ARTERIOSUS AND PULMONIC STENOSIS. HARRY GORDON and DAVID PERLA.

An instance is reported of subacute bacterial endarteritis (*Streptococcus viridans*) of the pulmonary artery associated with patent ductus arteriosus and pulmonic stenosis in which the valves showed no inflammatory changes. A boy, aged 13, with a congenital cardiac defect but in apparent good health developed an infection caused by *Streptococcus viridans* to which he succumbed within two and one-half months after onset. The clinical course was characterized by a septic fever, progressive weakness, secondary anemia and embolic phenomena in the spleen and kidneys. Autopsy revealed: (1) a patent ductus arteriosus and a congenital pulmonic stenosis with marked hypertrophy of the right ventricle; (2) subacute bacterial endarteritis of the pulmonary artery and the ductus arteriosus with large thrombotic masses almost occluding the lumen; (3) no involvement of the valves; (4) embolic lesions in the lungs, kidneys and spleen, and (5) acute arteritis and arteriolotis of the small pulmonary vessels.

The direct relationship of congenital defect to bacterial inflammation is discussed.

SPLENOMEGALY DUE TO PORTAL THROMBOSIS. SAMPSON J. WILSON and MAX LEDERER.

The patient, a boy, aged 2 years, had repeated severe gastric hemorrhages for a period of one year, necessitating many transfusions. His abdomen and spleen were large. A study of his blood showed it to be normal. He developed a peritonitis and sepsis before death. At autopsy the portal vein was found to be constricted just beyond the junction of the splenic and the superior mesenteric veins, and the esophagus showed varicosed veins at its lower portion. The spleen was the seat of a chronic splenitis. Microscopically, the portal vein showed a fibrous thickening in all three coats with a distorted elastica, which was destroyed in places: a typical phlebosclerosis.

Two additional cases with similar clinical courses were reported. Splenectomies were performed in these two cases, and both patients made uneventful recoveries.

The clinical course in portal phlebosclerosis, its etiology, pathology, differential diagnosis, prognosis and treatment were discussed and references to similar cases in the literature were given. The cardinal points on which the diagnosis should be made are the splenomegaly, the anemia, the signs of collateral circulation and the copious gastric hemorrhages which are followed by a diminution in the size of the spleen.

Photomicrographs of the lesion in the portal vein were shown.

DISCUSSION

A. A. EISENBERG: Under Dr. Hauswirth's guidance during the past six months, we have begun a systematic study of the veins. About six months ago there was admitted to the surgical service of the Sydenham Hospital a man, aged 32, who had been acutely ill for a week with edema of the feet, enlarged spleen and liver, and who complained of severe upper abdominal pain on the right and left side. His temperature was between 100 and 101 F. The surgical service to which he was admitted considered the diagnosis of an intra-abdominal infection, or the possibility of a hypernephroma on the left side, and Dr. Hauswirth paid especial attention to the superficial veins in his arms, which were found to be tortuous and hard. They felt as a sclerotic artery would feel. Because of these observations on the superficial veins, Dr. Hauswirth made a diagnosis of portal thrombosis, thinking the process was on the basis of a diffuse phlebosclerosis rather than an infection, of which there was no history. A superficial vein was taken out for examination. This proved to be a phlebosclerotic vein showing no evidence of inflammatory changes or degeneration, but rather a replacement fibrosis. The abdomen was opened, and no hypernephroma was found. The liver and spleen were greatly enlarged. The patient died on the third or fourth day after operation, and at autopsy, thrombosis of the portal vein, both of the main stem and of the portal branches, was found. A thrombus was also found in the left iliac vein, and an enlarged spleen, showing evidence of degeneration, was present. The sections from the portal vein showed what we thought were typical phlebosclerotic changes. In looking up the references to the condition, we found that phlebosclerosis occupied a place in pathology which might be called the stepchild of pathology. We could get little information about phlebosclerosis, the prominence being given to varicose veins, phlebitis, etc. We went to the earliest works and found that the incidence of phlebosclerosis pathologically was regarded as unknown. Etiologically, there is the opinion that it is a senile degeneration (Lobstein), while Sacks believed it to be a general disorder of nutrition, and it has also been regarded as an inflammatory condition (Fischer, Koestner).

These changes were found within the last six months in a rather small medical service; there were twenty-two cases in which the superficial veins, both in the arms and in the legs, especially around the ankles, presented the same macroscopic and microscopic picture. An interesting phenomenon is that they all seem to have a fairly uniform condition associated with them. Sixteen of our twenty-two patients gave a definite history of gastric ulcer; they all seem to have a heart

which is rather smaller than usual. On the basis of what we have seen so far, we believe that these changes are neither degenerative nor inflammatory. They seem to occur especially in persons between 30 and 40 years of age, only two patients being past 40, and the remaining ones between 28 and 40. They seem to be symptomless, and without any definite etiology. The extent to which they may be symptomless is shown by a case of Buerger's disease which we have in the hospital. We removed a piece of vein from the good leg, and that is completely thrombosed, showing the same fibrotic changes, subendothelial thickening of the intima and replacement fibrosis of the media. Only a week ago Dr. Hauswirth and I came across two articles written twenty-five years ago by Martin and Meekins and by Martin and Tull from Montreal. They examined children and adults, and claimed that they found phlebosclerotic changes in more than 30 per cent of their cases.

PAUL KLEMPERER: I think the importance of changes in the portal circulation causing splenomegaly cannot be overemphasized. During the last three years I have performed an autopsy in not less than six cases of splenomegaly in which the condition was laid to "Banti's disease," but was due to an embarrassment of the portal circulation. One of these cases showed an obstruction in the splenic vein alone; in the other five the portal vein was the main site of the lesion; there was occlusion in five cases, and merely a severe sclerosis of the portal vein in one case. The pathogenesis of changes in the portal system is simpler, I think, in children than in adults. In adults there is a multiplicity of causes which can cause such an occlusion of the portal vein, among which, of course, inflammation of the wall, thrombosis, and pressure from without play the main rôle. In children I would be inclined to consider mainly inflammatory changes in the portal vein as the cause of the subsequent either narrowing or complete thrombotic occlusion. That no history of an umbilical infection can be elicited in some of these cases, as in Dr. Wilson's case, seems to me not to be convincing proof that there was not one present. I remember cases, one particularly, in which I found even an acute suppurative phlebitis of the umbilical vein in a new-born child with characteristic abscesses in the liver without any clinical evidence of umbilical infection, though it was looked for. I think therefore that a milder infection of the umbilical vein can be easily missed but may lead, nevertheless, to a productive phlebitis of the portal vein.

I believe phlebosclerosis, particularly of the portal vein, is a condition which must have a definite histologic picture. Phlebosclerosis was first described by the early authors but was studied in detail by Borrman and later on by Simmons. I have studied a number of cases of phlebosclerosis, particularly in cirrhosis of the liver, and I have thought the changes in the vessel wall are similar to the changes in arteriosclerosis. One of the main features of a phlebosclerosis of the portal vein is the lamellation of the internal elastic layer; this I have seen definitely in cases of cirrhosis of the liver, and in the one case of splenomegaly I have mentioned before. My other five cases are divided and had different pathogeneses. During the last weeks I have again observed one case of thrombosis of the portal vein with canalization of the lesser omentum with so-called cavernomatous transformation. The patient in this case had a severe inflammation of the portal vein and of the superior mesenteric vein. The etiology was not clear. The patient had a four plus Wassermann reaction, but there were no other syphilitic changes. Simmons considered nearly every case of portal phlebosclerosis syphilitic in origin. I think this is exaggerated, and I cannot subscribe to it. The patient in this particular case had gangrene of one toe, which was clinically considered to be a thrombo-angiitis obliterans. One has to consider that thrombo-angiitis obliterans with its concomitant migrating phlebitis might be an etiologic factor in the causation of portal thrombosis. I should like to mention two more points. One receives frequently an enlarged spleen in the surgical pathologic laboratory from which the condition has to be diagnosed by the histologic picture alone. I think one peculiar feature is characteristic for splenomegaly due to embarrassment of the portal circulation, and that is the enormous increase in the sinuses of the spleen, the

hyperplasia of the sinuses, which one sees in these cases. During recent years Duerk presented the sinus hyperplasia as one of the true criteria of "Banti's disease." I do not think it is actually so. If there exists such an entity as "Banti's disease," the sinus hyperplasia is not one of its characteristic features. If there is no evidence of inflammation in the spleen and mainly the sinus hyperplasia, I should suggest an embarrassment of the portal circulation as the cause of the splenomegaly. One question remains in regard to the treatment in these cases. Take the instances in which one suspects an embarrassment in the portal circulation. If there is an embarrassment in the portal vein proper, I do not see any reason for a splenectomy. Perhaps one would prolong the life of the patient for a short while; perhaps one would shorten it, but if there is a definite evidence of occlusion of the splenic vein, I think splenectomy might be the rational treatment. Of course, a surgeon can never tell us whether or not there is any embarrassment in the portal circulation, because even with the best exposure, he does not reach the portal vein so well that he can definitely make a statement about its condition, particularly when the embarrassment is not due to actual thrombosis but only to phlebosclerosis. I have tried at the autopsy table to realize by palpation whether there was a change in the wall of the portal vein, and I could not do it in cases in which the microscopic examination revealed definite changes.

LOUIS HAUSWIRTH: Dr. Eisenberg did not emphasize the suggestion that we have made about the value of this particular work. It is more or less a rediscovery, so to speak, and the clinical significance is being studied. In one case a diagnosis of probable phlebosclerosis of the portal vein was made because the veins in the arms and legs were hard and almost completely closed; the veins had lost their normal color and looked like white cords. I call your attention particularly to that feature. Veins in the lower extremities are more frequently involved than the others. These veins, even at the time of excision, look like anything but veins. They are veins in which the lumen has almost completely disappeared. This particular patient had that condition in his superficial veins to an exquisite degree. The portal veins showed the same condition. The hepatic veins and the portal circulation likewise showed it. The iliac vein was a striking feature of the same lesion. What we want to emphasize is that this condition is not local; it is generalized. Just as the arteries do not give symptoms unless there is something added to the change in the vessel, so the veins give no symptoms unless infection or injury occurs which makes inflammation possible. Our patient had degeneration of the liver. All the veins took part in this general process.

We chose patients who had a peculiar type of build, more or less the asthenic type; somewhat cyanotic, with dropped and small hearts. These patients had ulcer of the duodenum or of the stomach. The possible causal relationship of these lesions is particularly what concerns us. In the first place, we know that the veins and arteries are thrombosed and fibrosed in cases of ulcer of the stomach and duodenum, and it is curious that this observation should not have attracted more attention. I said at the time that varicose ulcers of the leg resembled very much the ulcers one sees in the stomach and duodenum. At present, we have a boy, aged 19, with an ulcer of the leg of two and a half years' duration which will not heal, and the ulcer looks exactly as a peptic ulcer would look, without any tendency to healing. All his veins partake of the sclerotic nature. The point I wish to make is that these patients with digestive ulcers also show the same lesions in the veins. Another curious case was one of Buerger's disease of five years' duration in a man, aged 26, in whom the toes of one foot were gangrenous. There were no changes to indicate any lesion of the vessels in the apparently normal leg, and yet a large vein was completely thrombosed, the walls thickened and the thrombus completely canalized. There is nothing inflammatory about these changes in the veins, and it is only in the hope of arousing more interest that we want to emphasize the relationship of gastric and other lesions to a generalized phlebosclerosis. These cases occurred in young persons. In none of them do we find the characteristic changes of arteriosclerosis. We had twenty-two cases, and

in none of the excised veins did we find a condition analogous to arteriosclerosis, and we have therefore suggested the name phlebofibrosis instead.

MAX LEDERER: In relation to splenectomy, I do not believe that one is advised to treat the condition in the portal vein. Splenectomy is advised in order to prevent, if possible, the hematemesis which may occur, especially after the inflammatory process in the portal vein has ceased. It is conceivable that there may have been an inflammatory process with narrowing of the lumen of the vein, and therefore a portal insufficiency. By removing the spleen, that load is taken from the portal vein, and it is possible that these patients may survive because a great many of them do die from esophageal hemorrhage. Only two weeks ago I performed an autopsy in a case of syphilitic aortitis with marked interstitial changes in the lungs, and the right and left sides of the heart were hypertrophied. The subject had a spleen which weighed 350 Gm., adherent at the upper pole, with a thick vascular omentum. We thought at first that the splenic enlargement was due to the syphilitic process, and we found we could not enter the portal vein. It was represented by a fibrous cord, and only after a great deal of care and dissection were we able to pass a fine probe into the portal vein. I think this was one case due to syphilitic infection. In the literature syphilis, syphilis and syphilis is mentioned, the idea being that most of the cases are of syphilitic origin. I believe as far as the phlebosclerosis of the portal vein, or the pyelophlebosclerosis, as it is called, goes, that it may be a local condition. There is no evidence of any other change in the vein. I want to say in conclusion, which I think I can say without hurting the modesty of Dr. Klemperer, that the original diagnosis of the possibility of thrombosis of the splenic vein in the case reported by Dr. Wilson was made by Mrs. Klemperer.

CHICAGO PATHOLOGICAL SOCIETY

Regular Meeting, Feb. 10, 1930

HENRY C. SWEANY, *President, in the Chair*

THE STRUCTURE OF THE LUNG AND ITS IMPLICATIONS IN INFLAMMATION OF THE ORGAN. WILLIAM BLOOM.

Evidence is presented that the respiratory portion of the lung is primarily of mesenchymal nature and that the so-called alveolar epithelium and the non-nucleated plates probably do not exist. The respiratory portion of the lung reacts in inflammation like a purely mesenchymal tissue; that is, by the exudation of hematogenous exudate cells and by the much slower mobilization of pericapillary mesenchymal cells. Inflammation in this organ thus affords a denial of those theories which explain the presence of all of the exudate cells in inflamed tissues as being of purely histogenous origin. This subject will appear in more detailed form as a part of a chapter in Maximow's "Textbook of Histology."

SYMPTOMATIC AND PATHOLOGIC OBSERVATIONS ON ANIMALS INOCULATED WITH THE VIRUS OF POLIOMYELITIS. PAUL H. HARMON.

In tests to determine the susceptibility of a number of species of mammalia to parenteral inoculation of the virus of poliomyelitis, a sporadic incidence of suggestive symptoms and of suggestive cellular accumulations in the central nervous system was observed.

Virus, the potency of which was established by parallel inoculation into *Macacus rhesus* monkeys, when introduced into eighteen young rabbits by the combined intraperitoneal intracerebral method, resulted in the death of eight rabbits, and the appearance in three others of symptoms suggestive of prostration, convulsions

and transient motor pareses. Similarly, of seven young rabbits inoculated with normal monkey brain emulsion, none of which showed symptoms, two died.

When an emulsion of brain and cord removed aseptically from a number of rabbits which had died following inoculation of monkey passage virus was introduced into other rabbits (twenty-three in all) death followed in the second, third and fourth series of rabbits, thus simulating virus transfer from one generation to another. Eighteen of these rabbits died, and one showed suggestive symptoms. At the same time seven of nine rabbits inoculated with normal rabbit brain emulsion succumbed. None of these showed symptoms. It was shown, however, that no poliomyelitis virus was involved in these transfers, because rabbit brains of three different passages, in five instances, failed to induce poliomyelitis in monkeys, although inoculated intraperitoneally and intracerebrally in large quantities.

Over the same time, in thirty-eight uninoculated young rabbits, kept under similar conditions, twenty-one succumbed spontaneously, seven of which were observed to have died in convulsive seizures with symptoms identical with those seen in rabbits inoculated with virus. This correspondence in symptoms and mortality rate in the three groups of rabbits indicated that some other factor than the transmission of poliomyelitis virus from rabbit to rabbit was operative, which in all probability was miscellaneous intercurrent infections and infestations, since gross lesions of sufficient magnitude to have caused death were demonstrable in about 75 per cent. Among these lesions were disseminated and localized coccidiosis, tape-worm larvae infestations, bronchopneumonia with edema and hemorrhages into the lungs, and hemorrhages into the large bowel.

Microscopic lesions suggestive of poliomyelitis were found in the cerebrum and spinal cord, chiefly in the gray matter of four of forty-one rabbits inoculated either directly or in passage experiments with virus, and consisted of focal and perivascular accumulations of mononuclear cells. In the spinal cord there was often, in addition, a disseminated increase in cells in the gray matter. The most suggestive lesions were observed in the spinal cord of a rabbit which had shown no motor symptoms, but which was killed for examination. In a series of twenty-six uninoculated rabbits, dead spontaneously — with, or without symptoms — or chloroformed when apparently in perfect motor coordination, similar microscopic lesions were found in four. No perivascular collections of cells were found in the latter series, and neourophagocytosis was absent in all.

Of eleven dogs inoculated with poliomyelitis virus in three successive experiments, but two succumbed. At the same time, three of seven dogs that were inoculated with normal monkey brain emulsion succumbed. No symptoms were observed at any time in the animals that survived. Similar observations were obtained among uninoculated control animals. Postmortem examinations of the fatal cases showed bronchopneumonia of the lungs, while both in these animals and in many others that showed no effects following inoculation, no alterations were found in a carefully conducted search through many levels of the central nervous system.

Three of twelve young cats inoculated with virus succumbed, while among five controls receiving normal monkey brain none succumbed. No symptoms were observed in animals that lived. Gross pathologic examination, confirmed by microscopic methods, showed that when death occurred it was due to acute respiratory disease. Microscopic observations extended to many levels of the central nervous system, both in the cats dying from respiratory disease and in those killed following an uneventful five week observation period, failed to demonstrate changes.

Three diversified experiments with poliomyelitis virus on twenty-five guinea-pigs resulted in the death of thirteen of these animals. Among fifteen controls receiving normal monkey brain, three succumbed. In most of these, examinations showed that death was due to acute respiratory disease. Careful search in many microscopic sections of the central nervous system failed to reveal changes that might be interpreted as those of poliomyelitis.

Among thirty-two white mice inoculated with virus, ten succumbed in the five week observation period, while among thirty-one mice inoculated with normal

monkey brain, seven succumbed in a similar period. No symptoms suggestive of flaccid motor paralysis were observed in any of these animals. Gross pathologic observations established the cause of death in a majority of instances as due to acute respiratory disease.

Of four young pigs inoculated with massive doses of virus, none showed symptoms. Two young pigs receiving normal monkey brain showed no symptoms during the observation period.

Two of four lambs inoculated with virus developed suggestive motor incoordination, but this was due to the concomitant general prostration of pneumonia. No symptoms were observed in control animals. No alterations were found in the central nervous system of the affected animal.

Two of three calves succumbed following inoculation with virus, but no alterations could be demonstrated after a careful examination of the central nervous system.

Conclusions.—1. In attempts to transfer poliomyelitis to young rabbits, with virulent monkey passage virus, occasionally death, sometimes with suggestive motor symptoms, was observed. Young rabbits were shown to be markedly susceptible to intercurrent infections and infestations.

2. Convulsive seizures and other motor symptoms, as well as certain cellular changes in the central nervous system of rabbits inoculated with poliomyelitis, are nonspecific, identical symptoms and cellular infiltrations being observed both in rabbits inoculated with normal rabbit brain and normal monkey brain, and in uninoculated young rabbits.

3. Young dogs, young cats, guinea-pigs, mice, young pigs, lambs and calves do not develop any condition recognizable as, or related to, poliomyelitis following massive inoculation of virus of established pathogenicity for monkeys. Although a small proportion of inoculated animals succumbed, death is due, in the main, to acute intercurrent respiratory disease.

TUBERCLE-LIKE STRUCTURES IN HUMAN GOITERS. R. H. JAFFE.

In three nodose goiters and in one diffuse goiter with mild hyperthyroidism there were found in the removed thyroid tissue nodular structures composed of epithelioid cells and giant cells which resembled miliary tubercles. The origin of the cells could be traced to the follicular epithelium. The patients had no other signs of tuberculosis and made an excellent recovery. Tubercle bacilli could not be demonstrated in sections nor in the sediment obtained by dissolving a great many frozen sections with the aid of antiformin. There was no caseation in the nodules but much fibrous tissue about them.

The literature contains numerous reports on benign miliary tuberculosis of the thyroid. It is questionable, however, whether all these cases are really tuberculous, because in most of the reports there is no support of this diagnosis other than the microscopic similarity with miliary tubercles. The explanation is advanced that tubercle-like structures may result from noninflammatory involutional changes of newly formed or old follicles of the thyroid. These changes should be separated from true tuberculosis of the thyroid and from tuberculous goiters.

PULMONARY SIDEROSIS: TWO CASES WITH RETICULO-ENDOTHELIAL SIDEROSIS. MILTON G. BOHROD.

Two cases of pulmonary siderosis are reported; in one in which the dust was metallic iron, the black variety of siderosis was produced and ended in a florid tuberculous bronchopneumonia; in the other, in which the dust was iron ore, the siderosis of the red variety and the concomitant tuberculosis were of the fibrotic type and limited to the lymph glands.

In both there was a continuous attempt at excretion of the iron by macrophages which carried it into the alveoli, thence into the sputum, by excretion through the bronchiolar mucosa, or by transference to the lymph nodes. In the latter

event, the pigment either was deposited in the gland or was carried through the gland and reached the blood stream probably by way of the thoracic duct. Having reached the blood stream, the iron was deposited in the cells of the reticulo-endothelial system.

In addition to transportation there was gradual transformation of the iron. Only the soluble iron could be transported and was found intracellularly. In the black variety, where more silicon than iron was present in the lung, only the more soluble iron found its way into the blood stream. Insoluble iron was found extracellularly in dense fibrous tissue.

Where the accumulations of iron were greatest the amounts present were 8.9 and 7.9 per cent of the moist weights of the lungs.

The chemical examination of the sputum for iron may be an aid in the clinical recognition of siderosis.

DETORSION DEFECTS IN CONGENITAL CARDIAC ANOMALIES: REPORT OF THREE CASES, WITH AN ANALYSIS OF THE MECHANISM OF THEIR FORMATION. PHILLIP F. SHAPIRO.

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PHILADELPHIA PATHOLOGICAL SOCIETY

Regular Meeting, Feb. 13, 1930

BALDWIN LUCKE, *Presiding.*

INFLUENCE OF AGE ON ANTIBODY FORMATION. JULES FREUND.

Immunity conditions in very young and in adult animals differ considerably. This difference may invalidate conclusions formulated without taking into consideration the influence of age on immunity conditions.

It is known that hypersensitiveness to the intracutaneous injection of tuberculin is not transmitted from tuberculous female guinea-pigs to their offspring. This fact has been used as one of the chief arguments for the view that antibodies play no rôle in the intracutaneous tuberculin reaction. This argument is not valid in the light of the demonstration that the skin of young tuberculous guinea-pigs is quite refractory to the injection of old tuberculin during the first month after birth (*J. Immunol.* **13**:285, 1927; **17**:465, 1929).

In rabbits less than 20 days old, the formation of agglutinins against typhoid bacilli, of hemolysins against sheep cells and of precipitins against horse serum and egg white is strikingly less intense than in adult rabbits immunized in the same way. A definite Arthus phenomenon cannot be produced in young rabbits immunized with horse serum or egg white.

SYSTEMATIC ANALYSIS AND CLASSIFICATION OF SKIN TESTS. HERBERT FOX.

Contradictory and irregular skin reactions to the toxins and emulsions of streptococci in certain diseases such as arthritis, endocarditis and nephritis, dictated the need of analyzing the facts known about all the groups of skin tests. They are tabulated and analyzed numerically. They are found to fall into four rough groups, in which the following combinations are indicated:

Anaphylaxis, Schick and Dick tests fall together, tuberculin and typhoid are close but not identical, and animal parasites are numerically the same as the last two. These five are distinct from the natural serum, epidermal, pollen, drug and dust sensitivities. Food sensitivity belongs in an entirely separate group.

The inconsistency of the whole tabulation indicates a weakness in the knowledge of the mechanism; that the antigen and test are less important than the bodily mechanism, and that the constitutional basis is perhaps the most important of all.

It will be necessary to have a knowledge of normal cutaneous responses, capillary permeability, simple chemistry and physiologic chemistry before a real standard of skin response can be established. To this end, it may be possible to create a standard and norm per individual by the use of a previously standardized quantity of histamine at each injection for skin test purposes.

CRITIQUE OF THE BACTERIAL SKIN TESTS, WITH A SPECIAL REFERENCE TO STREPTOCOCCI. MACKINNON ELLIS.

The interpretation of bacterial skin tests is complicated by variations in technic and by lack of understanding of the mechanism. Technical differences may lead to complete disagreement of results. The conception of the toxic reaction has over-reached itself and has led to much misinterpretation.

A true toxic reaction must be neutralizable by immune serum, not passively transferable and thermolabile. Negatives are due to antitoxin and to individual hyporeactivity.

The Schick, Dick and *Streptococcus erysipelatis* reactions are apparently true toxic reactions, whereas the so-called toxic reactions of various streptococci fall better in a group with the tuberculin reaction. The typhoidin reaction and pseudoreaction to the Schick test also fall in this group.

Animal experimentation shows that rabbits can be made skin reactive by inoculation with streptococcus preparations. Desensitization can be accomplished by the intravenous method only. There are four kinds of response: normal, hypersensitive, immune and cachectic.

The relation of streptococcus skin testing to disease is discussed, but no conclusions could be drawn, since the underlying mechanism is not known.

LYMPHATIC PERMEATION STRIKINGLY ILLUSTRATED IN A CASE OF CARCINOMA PROBABLY PRIMARY IN THE THYMUS. GEORGE M. ROBSON.

Lymphatic permeation is commonly observed in carcinoma. The following case is of interest because it presents such a striking picture of this mode of dissemination. The case is also of interest because of the probable thymic origin of the tumor.

The patient was a white man, aged 53, admitted to the University Hospital because of cough, vomiting and difficulty in swallowing. He was blind in his right eye. The dysphagia was largely paralytic, and there were other evidences of paralysis of the tenth and twelfth cranial nerves. The duration of illness was about six months, with severe symptoms developing rapidly during the last few weeks of life. On admission the patient was dehydrated and emaciated, having lost 45 pounds 20.4 Kg. He passed rapidly into a semicomatose condition, and died suddenly a few days after admission.

At autopsy, 600 cc. of clear fluid was found in the right pleural cavity. The pericardial sac contained 620 cc. of bloody fluid incident to a fibrinosanguineous pericarditis. The mediastinum was extensively involved by a neoplastic process which extended to the pericardium, left lung and pleura. The thymus was moderately enlarged, weighing 35 Gm. It was firmer than normal. On section, the surface was found to consist largely of grayish-white tumor tissue, a number of fibrous trabeculae and adipose tissue. Its outline was preserved, and it could be separated from the surrounding structures with a fair degree of ease, although it was somewhat adherent. The surrounding tissue was infiltrated, with involvement of the lymph nodes, especially of a group around the hilus of the left lung and a chain extending up to the right side of the neck.

The tumor extended into the left lung as large cords of soft white material in dilated lymphatics about bronchi and blood vessels. On the pleural surfaces

it formed a network of white cordlike elevations outlining the lobules. Both layers of the pericardium were partially covered by fibrin and showed extensive infiltration similar to that seen in the left lung. Just back of the pericardial sac, between it and the lower portion of the esophagus, there was a layer of moderately firm, whitish tumor tissue 1.5 cm. in thickness. In the adjacent muscular wall of the esophagus, there were two nodules of similar appearance, the larger measuring 4 by 3 by 2.5 cm. The mucosa was intact and movable over both, but the larger nodule caused a partial compression stenosis. Two enlarged lymph nodes were seen in this region. The only noteworthy lesion below the diaphragm was a nodule 6 mm. in diameter in the left suprarenal gland.

The brain showed flattened convolutions. Everywhere, but especially over its base, the pia was thickened and whitish. On section 20, soft grayish-white tumors were found within the brain, varying from 2 or 3 mm. to 3 cm. in diameter. The larger ones were in the cerebellum. Most of these were obviously in continuity with the thickened meninges, representing an extension into the brain from the meningeal involvement in deep sulci.

Histologically the tumor everywhere was composed of a large epithelial type of cell with an abundant, slightly basophilic cytoplasm. These occurred in sheets masses or cords with very little or no stroma other than that of the preexisting tissue. Their nuclei were quite large and prominent. The chromatin in them was arranged into a coarse reticulum. Most of the nuclei contained large eosinophilic nucleoli. Mitotic figures were numerous. Nowhere was there any prickle cell formation or keratinization.

The thymus showed the most advanced type of involvement. Sections from it were composed of almost solid cellular tumor areas. The thymic tissue was reduced to small accumulations of lymphocytes, with occasional small adipose areas. Only occasional Hassall's corpuscles were observed. From the carcinomatous areas, strands of tumor cells extended into the surrounding tissue, usually in the lumina of definitely recognizable lymphatics, frequently accompanying blood vessels.

The invasion of the esophagus was limited to the muscularis and submucosa, and consisted of cords and strands of cells infiltrating between the bundles of muscle fibers, many of which were atropic. Necrotic changes were present in the central portions of the nodules. Many widely dilated lymphatics containing cords of tumor cells were present at the outer edge of the tumor in the muscular coat.

In the lung the tumor seemed to be confined entirely to the lymphatics, which were seen as thin-walled, endothelial lined spaces following bronchi or blood vessels and containing cords of tumor cells. They varied in size from that just wide enough to accommodate two or three cells, to several millimeters in diameter. In the larger ones, there was much central necrosis of the cells. The invasion seemed to be from the hilus toward the periphery of the lung, and in many places to reach the pleural surface. In the pleura there were similar widely dilated lymph spaces filled with partially necrotic tumor and producing the white network noted in the gross specimen.

Both layers of the pericardium were covered by a fibrinous exudate with beginning organization. It everywhere contained greatly dilated lymphatics filled with tumor cells. Here and there strands of these invaded the organizing exudate. At several points, strands of tumor cells accompanied the arteries deep into the myocardium.

The histology of the brain and meninges was spectacular. Everywhere the pia was infiltrated with cords, sheets, strands and masses of large epithelial cells. These followed the pial vessels as they descended into the brain substance, surrounding them with cylinders of tumor cells, evidently contained in the Virchow-Robin spaces. This process was most marked in deep sulci filled with tumor. Tumor masses within the brain connected with those in the meninges in this manner. The right optic nerve showed an extensive infiltration beneath its sheath, thus accounting for the blindness of that eye.

Book Reviews

BACTERIOLOGY, ESPECIALLY DETERMINATIVE BACTERIOLOGY. By PROF. DR. K. B. LEHMANN and PROF. DR. R. O. NEUMANN. Seventh completely revised edition. Volume 1. Parts A and B, Technique and General Determinative Bacteriology. Part C, Atlas. Translated by Prof. Dr. Robert S. Breed. Price, $7.50. Pp. 172, with 3 text figures and 65 plates (63 in color). New York: G. E. Stechert & Company, 1930.

LABORATORY MANUAL OF BACTERIOLOGICAL METHODS. By the same authors and translator. Price, $1.50. Pp. 103, with 3 text figures and 5 colored plates. New York: G. E. Stechert & Company, 1930.

During more than thirty years, many beginners in bacteriology have been guided by the atlas and manual of determinative bacteriology of Lehmann and Neumann; specialists have used it as a reference book, and taxonomists have fought over the system of classification of micro-organisms employed in it. This English translation of the seventh edition will undoubtedly extend the battle-line of taxonomic argument, place Neumann's beautiful pictures of bacteria in the minds of new beholders and continue to guide — and, unfortunately, also misguide — beginners.

The fundamental purposes of the first edition of 1896 and of succeeding editions have been both taxonomic and technical. The colored plates illustrating the volumes have supported both of those purposes, serving as checks on the results of procedures and as a vivifying illumination of the text. While many of the pictures of black medium with colonies or stab cultures crusted on the background seem stiff and unnatural, they have been and remain the unique feature of the work. They have lost none of their brilliance in this latest reproduction.

In the taxonomic field, Lehmann's influence has been on the side of conservatism. An inspection of this first volume shows that he has maintained that position. Recognizing the variability of bacteria, he has combined species and has created few new ones. He cautions the bacteriologist to remember that "variability of bacteria produces many difficulties for bacteriologists. For this reason he should never christen an organism as 'new' until he has determined that it is absolutely necessary." Classifications would be greatly simplified if the industrious nominators in bacteriology would heed this admonition. In this volume, Lehmann has used neither Bergey's classification nor his nomenclature. He uses trinominals like *"Bacterium tussis-convulsae,"* designates the pneumococcus *"Streptococcus lanceolatus"* and gives the term bacillus to both spore-bearing aerobes and anaerobes. In addition, there is no evidence in this first volume that the significant modern studies of bacterial variation have been considered at all. While the first volume provokes these comments on an inferential estimate of the author's point of view, it is inadequate to serve as a basis for the examination of Lehmann's manner of dealing with these questions. It will be more appropriate to postpone that inquiry until the appearance of the forthcoming translation of the second volume, in which these matters will be considered in detail.

If one limits the examination to the specific content of the first volume, one discovers by the date of the preface, which is 1926, that the "seventh completely revised edition" is already in its fifth year. Neumann was unable to collaborate in the preparation of this edition, and when the work was made ready for the press in 1926, Lehmann was assisted by Dold, Süssmann and Haag. As these authors cite only a few references to the literature later than 1922, it is to be anticipated that much of the newer work in bacteriology will not be found within

the covers of this volume. Almost all the references are to publications in German. Many formulas of the habitually used mediums and well tried stains are given, and the fundamental steps to be taken in isolating and identifying bacteria are set forth in order. The procedures are of unquestionable utility, but the descriptions of them are not always as definite and precise as would be required by one who wishes to follow them exactly. References to the original sources are given in many cases, but there is little reference to the changes and improvements that have occurred with the passage of years. Many useful procedures are omitted, and in this volume serologic procedures are mentioned only by title, as they are to be described in volume 2. To a worker in this age of the facile hypodermic syringe, the setting is given an antique air when it is recommended that animals be "inoculated with a platinum loop."

The separate Manual is a verbatim reprint of the first 103 pages of the first volume and the first five colored plates. References to other plates in the first volume and to procedures in the second volume are given in a way that makes it seem unlikely that this section was intended to be exploited as a manual. It is doubtful whether it can, with fairness, be said to contain a "description of bacteriological technic commonly used in German bacteriological laboratories." In any case, manuals are usually such local directories that those which serve general needs are rare. The manuals may agree in general principles, but they vary according to the practices of the chiefs of laboratories of states, cities and schools. This manual has not the qualities of universality. It will be of little use to a bacteriologist in this country. Some of the advice to the beginner is actually misleading, as, for example, in the "Detailed example showing how bacteria are identified," on page 99. The case chosen for study is one of "eczematous conjunctivitis." It is said here that the finding of gram-negative, biscuit-shaped cocci and small, thick, regular-shaped gram-negative rods in the smear of the exudate "indicates that the cocci are gonococci and that the rods belong to the colon group." In fact, as there are often other gram-negative diplococci and rods in conjunctival exudates, this observation indicates and suggests more possibilities — and probabilities — than this bit of guidance for the beginner would admit. In paragraph *c* of this section, it is said to the beginner that "tubercular organisms" are excluded by the failure to find them in an acid-fast stain of the exudate of this eczematous conjunctivitis. The lamentable results of false conclusions have shown that the categorical negative is inappropriate in reporting smear diagnoses of exudates.

The translation remains to be considered. Opinion of the rendition of the German into English fluctuates with the sections read, as there is some variability. In many sections, the translator has produced a smoothly-flowing English, precisely presenting the meaning of the difficult technical German. In others, the construction remains somewhat Teutonic and there are mistranslations of words and phrases. "It is even more certain to sterilize in an autoclave," and "If colonies appear only as one or two" are examples of the few inverted constructions. Examples of mistranslations are "entire" for "glatt," as in the sentence, on page 102, "entire or very finely granular," where the description is that of a smooth or finely granular colony edge. The reviewer is not an expert in either the German language or the German comestibles. His curiosity, however, was aroused when he found on page 36 that pile worms (*Teredo*) may be used in place of lean meat in preparing a certain medium. The German word is "Pfahlmuscheln," and *Teredo* does not occur in the German text. Dictionaries sanction the translation ship-borer for Pfahlmuscheln. But it seemed such a laborious undertaking to dig the worms out of water-logged, worm-eaten timber in order to use them as a substitute for a piece of lean meat that the reviewer sought the advice of those who might have engaged in this sort of fishing to provide food for phosphorescent bacteria. He is informed by those who have eaten Pfahlmuscheln that the succulent animal referred to is the mussel, *Mytilus edulis*,

and not the burrowing *Teredo*. Twice the translation relapses into German, with an "und" and a hybrid "prezipite."

As the book was printed in Germany, it is remarkable that so few words of this kind are found, and that there are only two or three typographic errors in the volume. The proof reading was done with great care. The good paper used, the clear typography and the excellent reproductions of the colored plates exhibit the attention to detail which the publisher devoted to the production of the volume.

Respect for the great and beneficial influence which Lehmann and Neumann, and their book, have had on systematic bacteriology, homage for Dr. Lehmann, who even after his seventieth year is still carrying on the struggle to lift bacteriology into the position as a science, and a sympathetic regard for the labor that has been devoted by the translator to the service of bacteriologists have made the writing of this review painful. With reluctance, the reviewer is forced to conclude that volume 1 of this work will be of little use to bacteriologists in this country.

S. BAYNE-JONES.

THE PATHOLOGY OF THE EYE. By JONAS S. FRIEDENWALD, M.D., Associate in Pathological Ophthalmology at the Johns Hopkins University. Price, $4.50. Pp. 346, with 253 figures, mainly photomicrographs from the Pathological Collections of the Wilmer Ophthalmological Institute and the Army Medical Museum, by Helenor Campbell. New York: The Macmillan Company, 1929.

This book should interest all who are concerned with the study of the diseases of the eye. It is not voluminous yet touches all important ocular disturbances. The book will be particularly valuable to the practitioner because the relation of clinical appearances to anatomic changes is emphasized on the basis of a study of ocular diseases through many years. It is well illustrated, and helpful references to the literature are given. The introduction reviews concisely the structure, physiology and pathologic anatomy of the eye and adnexa. Inflammations of the globe are then discussed. Excluding specific inflammations such as tuberculosis, syphilis and sympathetic ophthalmia, the inflammatory disorders of the eye are the most difficult to systematize. The author divides these into two categories, panophthalmitis and focal lesions of an acute or subacute character. One wonders if this division into general and focal types of ocular inflammation does not confuse more than it simplifies. The author does not distinctly differentiate the cellular changes of these inflammations as Fuchs does. The specific inflammations (tuberculosis, syphilis and sympathetic ophthalmia) are handled so well that the reader cannot but wish that space had permitted the author to write more fully about them. There is recorded an interesting observation of a myeloid type of infiltrating wandering cell in the eyes of stillborn syphilitic infants. The chapter on injuries is good. The knowledge of radiant energy is briefly but well presented. The introduction of a third form of glaucoma, subacute, seems superfluous and confusing. The different theories of glaucoma are briefly discussed, including the view now held regarding the relationship between the intra-ocular blood vessels and the aqueous humor.

Much may be said in favor of the author's grouping of clinical disorders of the eye into associated pathologic conditions rather than according to the usual anatomic arrangement. The grouping may give a better conception of those disorders which affect, in their progress, more than one structure of the eye. Glaucoma must always be taken up in this way, and the intra-ocular inflammations, both specific and nonspecific, are perhaps better so handled. The author's review of senile changes and arteriosclerosis in one chapter is appropriate. Injuries of the eyeball, hereditary anomalies and diseases of the eye, as well as tumors of the eye, lend themselves well to groupings involving all parts of the eye. Nevertheless, there is little question that pathologic anatomy as well as practical ophthalmology can be taught more systematically by adherence to the anatomic basis.

EDEMA AND ITS TREATMENT. By HERMAN ELWYN, M.D. Price, $2.50. Pp. 182. New York: The Macmillan Company, 1929.

In this book one sees something of a reaction against the prevailing tendencies to put the problem of edema on a purely physicochemical basis solvable by test tube experiments with water and membranes, for Elwyn visualizes edema as something far more intricate and vitalistic. He starts out with a brief consideration of some of the prevailing theories of edema and water regulation, pointing out their shortcomings, and emphasizing the view that the maintenance of blood volume and water concentration is a vegetative function requiring adaptive regulatory mechanisms that have been developed phylogenetically. A discussion of the methods used by the body to keep the total blood volume and the water concentration of the tissue approximately constant leads to the conclusion that for such a complicated exchange process some central regulatory mechanism must necessarily exist. Since for other vegetative functions, the center of regulation seems to lie in the subthalamic and hypothalamic areas, working through the sympathetic and vagus, a similar center closely related to a center for salt metabolism is assumed to exist. It is assumed that discharges from this center, under the stimulation of changes in the water concentration of the blood passing through the center, cause alteration in a "constellation of electrolytes at the intersurface of the two phases in the colloidal system of the cell," which in turn affects the water content of the colloids.

Obviously this is a highly speculative theory which piles up more unknowns and undemonstrables as explanation of the original unknown, but it nevertheless has value as emphasizing lines of thought not generally adequately followed by students of the problem of edema. That central influences may modify water metabolism is proved by the polyuria that results from lesions in the hypothalamic region, and hence one cannot leave out of consideration the influence of the central nervous system in discussing edema, as has too generally been done. Elwyn has brought together many interesting lines of evidence on which to build up his theory, and has made a distinct contribution to the literature of the fascinatingly involved subject of edema even though the presentation at times seems to have metaphysical qualities.

CLINICAL ASPECTS OF VENOUS PRESSURE. By J. A. E. EYSTER, B.Sc., M.D., Professor of Physiology, University of Wisconsin; Associate Physician, Wisconsin General Hospital. Price, $2.50. Pp. 135, with 7 illustrations. New York: The Macmillan Company, 1929.

As its title suggests, this monograph deals with rather a neglected phase of cardiovascular disease. The reviewer cannot agree completely with the author's concepts of the dynamics of heart failure. Nevertheless, the technic, normal controls and standards in various clinical disorders constitute an important addition to the diagnostic and especially the prognostic armamentarium of the clinician. The book emphasizes that alterations in the venous pressure commonly precede other peripheral manifestations of altered compensation of the heart. Although the book is meant primarily for clinicians, its fresh points of view make it worth reading by pathologists.